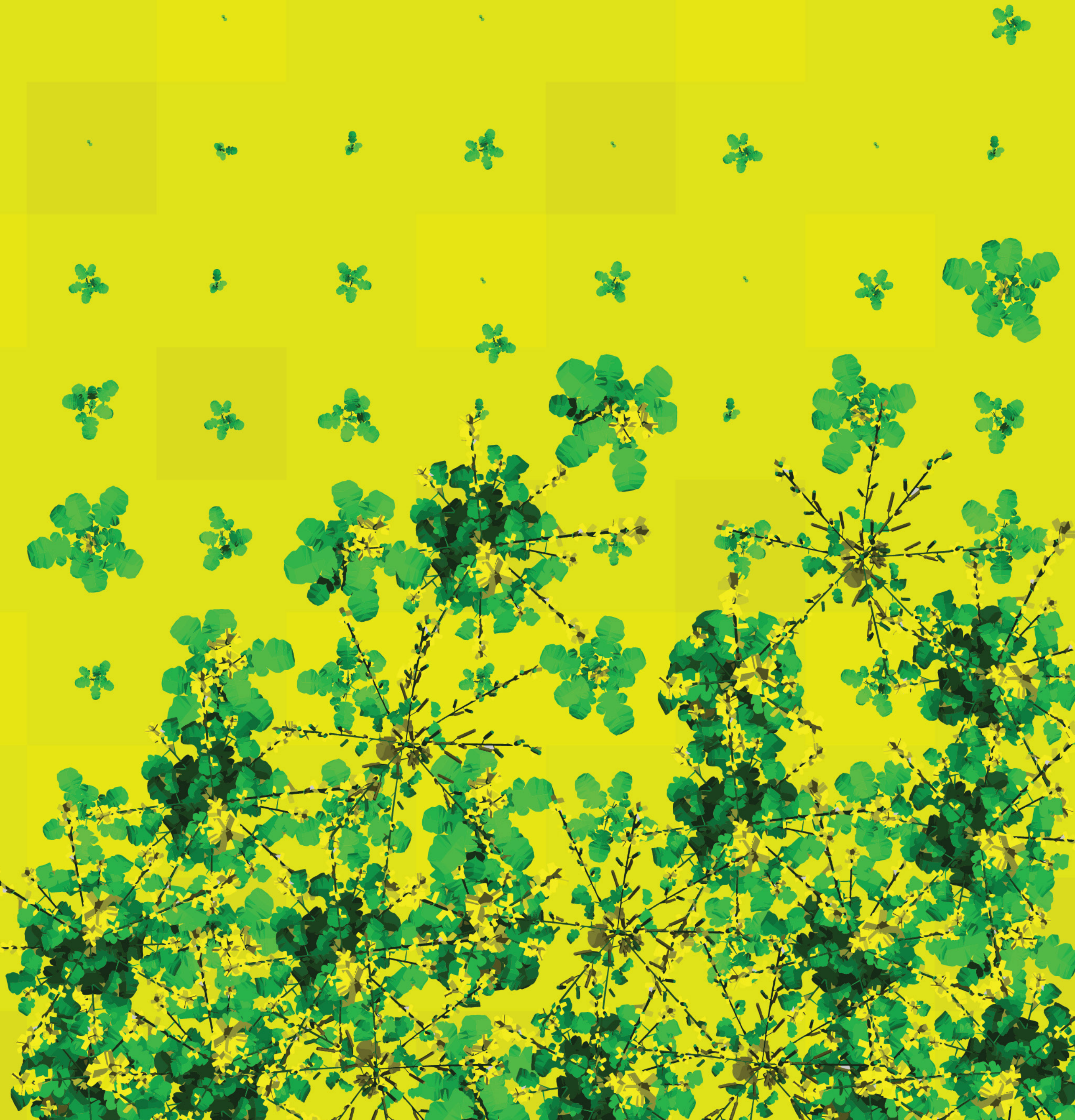
Phenotypic plasticity in oilseed rape in response to resource availability

Marcelo Labra Fernández



Propositions

1. A better understanding of phenotypic plasticity in oilseed rape is required for improving yield.
   (this thesis)

2. Seed weight is not a conserved trait.
   (this thesis)

3. The Multi-Layered Safety model implemented in the Netherlands for flood protection is a promising tool to be adapted in Chile against tsunamis.

4. Human welfare is a pre-requisite for farm animal welfare.

5. The proportion of PhDs per country is not an accurate indicator of scientific development.

6. Implementing a bike-friendly system requires a population educated with a bike-friendly mentality rather than costly infrastructure.

Propositions belonging to the PhD thesis, entitled:

Phenotypic plasticity in oilseed rape in response to resource availability

Marcelo Labra Fernández

Wageningen, 24 August 2020

Phenotypic plasticity in oilseed rape in response to resource availability

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This research was conducted under the auspices of the C.T. de Wit Graduate School for Production Ecology and Resource Conservation

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Thesis
submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. Dr A.P.J. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Monday 24 August 2020
at 1:30 p.m. in the Aula.

Marcelo Labra Fernández
Phenotypic plasticity in oilseed rape in response to resource availability, 182 pages.

PhD thesis, Wageningen University, Wageningen, NL (2020)
With references, with summary in English

ISBN: https://doi.org/10.18174/526714
DOI: 978-94-6395-462-4

Abstract

Over the last 50 years, seed production of oilseed rape (*Brassica napus* L.), the source of one of the healthiest edible oils for human consumption, has increased globally, reaching 71 million tonnes in 2017. However, food security will be challenged by the growth of the world's population, reaching 9.7 billion people in the next 50 years, increasing the demand for food, vegetable oils for human consumption, feed, and biofuel. Therefore, new strategies are urgently needed to satisfy the growing demand for oilseed rape, and one such strategies revolves around making use of plasticity in oilseed rape. The overall objective of this thesis was to evaluate the plastic response of oilseed rape to environmental factors and agronomic practices by identifying the main drivers and mechanisms underlying plant C and N distribution, with emphasis in the interaction between plant C status with plant architectural traits and physiological traits associated with seed yield. In this study, N fertilizer supply determined N content per unit of leaf mass (N_{mass}), while it played a subordinate role in altering N per unit of area (N_{area}), which was the main determinant of C assimilation. The rate of photosynthesis per unit of leaf area was strongly correlated with N content per leaf area (N_{area}) through an asymptotical relationship. N_{area} was more closely associated with leaf mass per unit of leaf area (*LMA*) than with N_{mass}. *LMA* was affected by a significant interaction between N supply and plant population density. Such an interaction plays an important role in the discrepancies observed in literature. Leaf area was affected by N supply, with important effects on light distribution and C assimilation at crop level. Indeed, the interaction between N supply and plant population density influences the leaf area index, which determines the proportion of light intercepted by the plant canopy.

The role of N supply as determinant of plant source-sink status (which is, in turn, the main determinant of branch outgrowth and silique production) was evaluated by combining experimental and Functional-Structural Plant (FSP) modelling approaches. To evaluate the role of N supply on branch outgrowth, an FSP model was adapted for oilseed rape. Branch and silique outgrowth were implemented as dependent on the plant source/sink ratio status, mainly driven by light interception. Since the numbers of branches and siliques per plant simulated by the FSP modelling approach were similar to the measured numbers, the hypothesized mechanism that the number of siliques is determined by the source/sink ratio, which in itself is affected by the number of branches, was confirmed. Additionally, the hypothetical limits of oilseed rape plants to produce siliques under a simulated branch inhibition were also explored.

The results suggest that the main raceme of an oilseed rape plant has the potential to develop a similar number of siliques as a plant with several branches. However, to achieve this full compensation, the model assumed several phenomena that are not very realistic, highlighting the importance of branching for yield.

It is assumed that resource limitation has a negative effect on plant productivity, but the responses of the seed yield components, such as the number of seeds per unit area, single and average seed weight and quality traits, to source limitation at flowering have been scarcely assessed in oilseed rape. In this study reductions in seed number per area in response to shading were due to reductions in both the number of seeds per silique, and the number of siliques per area. Surprisingly, the negative effect of shading on seed number per area was fully compensated by an increase in thousand-seed weight ranging from 47 to 61%, with increases in single seed weight up to twofold compared with the treatments under full sunlight and without affect oil and protein concentration. The sensitivity of the main seed yield components that account for variation in seed yield of oilseed rape in response to different environmental factors, was addressed using datasets collected from experiments performed in the Netherlands and Chile. Variations in seed yield per unit area were mostly explained by the number of seeds per unit area, while seed yield per plant strongly associated with variations in the number of branches per plant, number of siliques per plant and number of seeds per plant, while yield per silique was explained by the number of seeds per silique. But this does not mean single seed weight is a conserved trait, taking into account that under source limitation at flowering single seed weight was increased up to twofold compared with the treatments under full sunlight. This study hints at the necessity of re-evaluating the paradigm assuming that seed number per unit area is the main source of variation of seed yield, while seed weight is conserved, and suggests that the sensitivity of plant traits to environmental factors is not static but dependent on the crop, the type and timing of source limitation. By evaluating plant source and sink formation, and their interaction with plant architectural traits in response to environmental factors and agronomic practices, this thesis provided important information to improve the understanding of the mechanisms underlying yield formation and to define potential breeding targets for improving seed yield in oilseed rape.

Keywords: Functional-structural plant modelling, branching, *Brassica napus*, plasticity, seed weight, source-sink.

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Chapter 1

General introduction

1.1. Oilseed rape production and challenges for the XXI century

After the decade of 1970 the availability of carbohydrates, proteins and fats for human consumption was increased by an increase in cereal and oil crops production (Khoury et al., 2014). Oilseed rape (*Brassica napus* L.), source of one of the healthiest edible oils for human consumption (Momoh et al., 2004) and the third most consumed oil after soybean and palm oil, has increased its global production globally reaching 71 million tonnes in 2017 (FAOSTAT, 2020), partially powered by the rising demand for biofuels (Rondanini et al., 2012). At the year 2050 the world population will reach 9.7 billion people, challenging both food security and the production capacity of biofuel needed by such population (Ray et al., 2013; United Nations, 2019), in a scenario of limited arable lands, water, nutrients, energy scarcity and environmental degradation (Fan et al., 2011). Therefore, future attempts for improving crop productivity should also consider a more efficient use of resources (Fan et al., 2011; Sharma and Bali, 2018; Stahl et al., 2019), aiming to a more sustainable agriculture.

Since the expansion of cultivated area does not seem to be a large-scale sustainable strategy (Fan et al., 2011), yield improvement by plant breeding and by optimizing agronomic practices, appears as the most feasible way to continue increasing crop production of oilseed rape in the coming decades (Beddington, 2010; Rondanini et al., 2012). An example of this are the yield gains in a range between 0.6 and 2%, observed in the major producing countries such as China, Canada, India, France and Germany between the years 1991 and 2010 (FAOSTAT, 2015). This trend was strongly associated with genetic gains and the increase in agronomic inputs (Kirkegaard et al., 2016).

Historical records of rapeseed yield between the years 1970 and 2009 were analysed in twelve countries (Argentina, Australia, Brazil, Canada, Chile, China, France, Germany, India, Poland, the United Kingdom, and the USA) representing a wide range of environments and farming systems (Rondanini et al., 2012). Most of the evaluated countries showed increases in yield at rates in a range between 15 and 40 kg ha^{-1} $year^{-1}$. But in some of these countries, clear evidences of a stagnation in yield gains were observed during recent years. For example, the USA did not show significant yield increase since the 1980s, as well as the UK and Brazil, where the levelling-off has been evident from 1984 and 2000, respectively. Another study (Peltonen-Sainio et al., 2007a) showed a lack in yield gains in Finland, the northernmost region supporting large-scale oilseed rape, where the levelling-off are evident from 1990 and onwards. The stagnation in yield gains in these countries has been attributed to an inadequate combination of crop management factors such as lower N fertilizer input compared with previous decades, the use of short crop rotations and minimal cultivation (Berry and Spink, 2006). While in Northern European countries, the use of oilseed rape genotypes sensitives to high temperatures during seed filling has been also associated with reductions of seed yield (Peltonen-Sainio et al., 2007a). This information suggest that new strategies are urgently needed to overcome such levelling-off in yield progress and to satisfy the growing demand for oilseed rape.

Identifying plant traits associated with high agronomic performance (Foulkes et al., 2011; Thurling, 1991), and a better understanding of the mechanisms underlying the plant responses to environmental factors (Hikosaka et al., 2016; Ren et al., 2017), are key to develop high yielding cultivars and to optimize agronomic practices in agriculture.

1.2. Phenotypic plasticity of oilseed rape

Phenotypic plasticity is the ability of a genotype to modify its phenotypic expression in response to the local environmental conditions (Bradshaw, 1965). Traditionally, plant scientists have focused on breeding for crop varieties with a superior agronomic performance in a specific environment (Donald, 1968; Sadras, 2007), concept known as crop ideotype. The search for such an ideotype has focused the attention on morphological traits resulting of physiological processes to create cultivars with high agronomic performance for specific environments (Foulkes et al., 2011; Thurling, 1991). But environments are highly heterogeneous in space and time (Valladares et al., 2007), so plants must acclimate to variations in light intensity, air temperature and water vapor deficit (Pedruzo-Bagazgoitia et al., 2017; Sikma et al., 2018), as well as to physical and chemical soil properties (Tuffour et al., 2016; Vasu et al., 2017). Thus, the crop performance depends on the magnitude in which environmental factors and agronomic practices affect morphological and physiological traits (Dechaine et al., 2014; Sultan, 2003), determining the source and sink activity of plant organs.

In general terms, source organs are those plant organs in which net fixation of carbon (C) dioxide occurs, while the sinks are the organs where assimilates are stored or used (White et al., 2015). In oilseed rape, the leaves are the main sources of assimilates and their photosynthetic capacity and morphology can vary in response to environmental factors such as variations in nitrogen (N) fertilizer supply or leaf light environment (Bloomfield et al., 2014;

Gammelvind et al., 1996), affecting the formation of sink organs (Jankowski et al., 2016; Rondanini et al., 2017). Stem, branches and flowers are plant sinks, while siliques are initially a net source and then become net sinks. Siliques can make an important contribution to total assimilate production because of their photosynthetic activity (Gammelvind et al., 1996; Muller and Diepenbrock, 2006). However, from seed filling onwards, siliques also turn into strong sinks, because of the seed formation and subsequent biosynthesis of lipids in the seeds (Lambers et al., 2008), causing a strong competition for assimilates among different sinks. This can have important effects on seed and oil yield.

Plant architecture is defined as the three-dimensional organisation of the plant body (Reinhardt and Kuhlemeier, 2002) and one of the most important architectural traits of oilseed rape is stem branching pattern. The number, size and spatial distribution of stem branches are key determinants of plant source/sink ratio during the reproductive stage (Jullien et al., 2011), biomass allocation, number of siliques per plant (Pinet et al., 2015) and number of seeds per silique (Wang et al., 2011), suggesting that the agronomic performance of oilseed rape is determined by the interaction between stem branching and plant source/sink ratio.

Identifying the main determinants of source and sink formation, and their interaction with plant architectural traits, can provide important information to improve the understanding of the mechanisms underlying yield formation and to define potential breeding targets for improving seed yield in oilseed rape.

1.2.1. Nitrogen and light distribution inside the plant canopy during vegetative stage

A key factor in the success of food production during the last century was the intensification in the use of N fertilizers in agriculture. But the use of large amounts of N in agriculture has also led to negative environmental effects such as the eutrophication of freshwater (Cassman et al., 2002; Sharma and Bali, 2018) and losses in diversity and functioning of the bacterial, animal, and plant ecosystems (Tilman et al., 2002). Oilseed rape is a crop characterized by its high requirement of N input, because of both a low N-use efficiency and a relatively low N harvest index of around 50 % (Gammelvind et al., 1996; Schjoerring et al., 1995). Optimizing the balance between N fertilizer input and plant population density, two of the most important agronomic practices in oilseed rape production (Li et al., 2014; Ren et al., 2017), is key for a more efficient use of N fertilizers, while maintaining high production levels.

Variations in plant population density determine the penetration of photosynthetically active radiation (PAR) into the canopy, while the increase in N fertilizer supply is associated with leaf expansion, but not with photosynthesis per unit leaf area in oilseed rape (Gammelvind et al., 1996). A positive correlation between the light saturated rate of photosynthesis and leaf N content has been observed in several plants (Evans, 1989; Field, 1983). Despite the close relationship between N and C distributions, the ratio between the rate of photosynthesis and leaf N content, the photosynthetic N-use efficiency is not constant (Poorter and Evans, 1998). The relationship between leaf N content and photosynthetic rate per unit leaf surface has been investigated in a range of different plant species (Bertheloot et al., 2008; Dreccer et al., 2000; Field, 1983; Hikosaka, 2016; Hirose, 2005; Li et al., 2013; Monsi and Saeki, 2005), but not so far in crops like oilseed rape (Gammelvind et al., 1996).

In plant canopies, leaf N distribution has been shown parallel to the light distribution in an exponential decline of both over the canopy depth (Fig. 1.1a), explained by the Beer`s law (Hirose, 2005; Monsi and Saeki, 2005), which agrees with the optimal distribution of leaf N to maximizes C assimilation and crop productivity (Dreccer et al., 2000). In these approaches N and light are considered as distributed in vertical layers, being implicitly treated as clusters of independent tissues acclimated to their local light environment in the canopy (Bertheloot et al., 2012). But because plant canopies are characterized by a three-dimensional spatial heterogeneity, the absorption and distribution of resources not only follow a vertical pattern, but also a horizontal one (Caldwell, 1987).

A three-dimensional approach is particularly important when analysing N and light distribution in crops grown under a high intra-specific competition like oilseed rape (Fig. 1.1b). Variations in plant population density and N supply alters the light environment inside the canopy causing variations in leaf morphology (Gammelvind et al., 1996), or plastic responses of architectural plant traits like stem branching pattern (Hu et al., 2017) (Fig. 1.1c). These plant responses can affect light distribution inside the canopy, with potential effects on C assimilation and plant productivity. A better understanding of the regulatory mechanisms underlying the interaction between N and C distribution in dense canopies, is the point of departure to understand their role in seed yield formation.

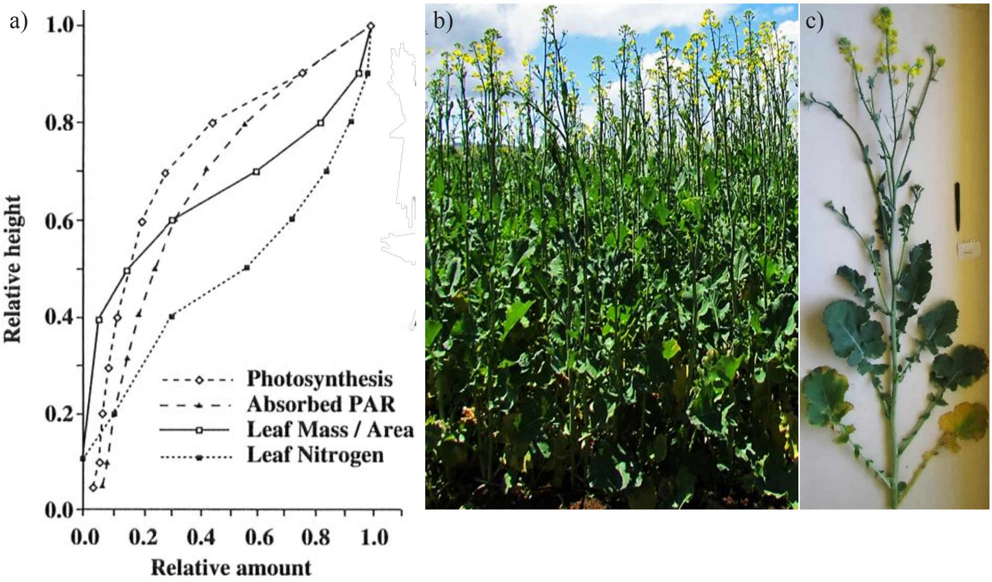

Fig. 1.1 a) Leaf photosynthesis, PAR absorption, leaf mass per unit area and leaf N content along the canopy height for herbaceous plants (Goetz and Prince, 1999). Pictures of b) canopy profile and c) single plant of oilseed rape, were taken from experiment performed in Chile 2014.

1.2.2. Role of nitrogen availability in branch and silique formation

Stem branching is one of the most characteristic traits of oilseed rape. The number, size and spatial distribution of branches determine plant architecture, crop architecture and source/sink dynamics (Jullien et al., 2011; Pinet et al., 2015), with important effects on plant and crop productivity. The number of branches per plant depends on the number of axillary buds along the stem that broke their dormancy (Wang and Li, 2008).

There are several factors influencing bud outgrowth, but one of the most studied ones is the PAR, which by its effect on leaf photosynthesis and plant C assimilates status determines branch outgrowth (Leduc et al., 2014). Environmental signals also play an important role in branch outgrowth, such as the red: far red ratio, a signal that indicates the presence of surrounding vegetation and triggers or suppresses avoidance responses in plants (Ballaré et al., 1990; Rondanini et al., 2014). Internal plant sugar signalling, in interaction with hormones such as auxins, strigolactones and cytokinins (Domagalska and Leyser, 2011; Rameau et al., 2015), is also closely associated with bud outgrowth.

Studies evaluating the effects of C availability and sugar signalling on bud outgrowth, should consider that all these signals interact with N signalling pathways (Osuna et al., 2015). Several studies show an association between stem branching and N fertilizer input in oilseed rape (Jankowski et al., 2016), but also in other crops (Longnecker et al., 1993; Sangoi et al., 2011; Vos, 1995), suggesting that N is important for branch outgrowth. It is also known that N is associated with C assimilation (Hikosaka et al., 2016) and meristematic activities (de Jong et al., 2014), but there is no evidence of a direct role in bud outgrowth (Rameau et al., 2015) and no studies have been carried out about this on oilseed rape.

During the window of time when the number of branches is defined, the numbers of reproductive organs like flowers and subsequently siliques, are also defined (Zhang and Flottmann, 2016). The number of siliques per plant is closely correlated with plant C status and sink strength between the stages of budding and beginning of silique development (Pinet et al., 2015; Wang et al., 2011; Zhang and Flottmann, 2016). Therefore, it is possible that organs such as branches, flowers and siliques are in strong and constant competition for assimilates during their development, but the magnitude of this competition should be investigated.

1.2.3. Interplay between seed number and seed weight in response to assimilate limitation at flowering

In the previous three decades, the primary focus for yield improvements was through increasing seed number per area (Diepenbrock, 2000; Gomez and Miralles, 2011; Peltonen-Sainio et al., 2007b), while seed weight did not change (Peltonen-Sainio et al., 2007b). The number of seeds per unit area and seed weight are two of the main yield components of oilseed rape, and the latest to be set during the plant life cycle (Mitchell, 1970). The time window during which seed number and single-seed weight are established are known to overlap around flowering, but to different degrees depending on the particular crop species (Calderini et al., 2001; Yang et al., 2009), period when plants allocate their assimilates to their reproductive organs setting the potential sink strength (Andrade et al., 2005).

Based on the assumption that “each organism has a critical stage in which their energy should be invested into maximizing their potential offspring”, Smith and Fretwell (1974) suggested that plants distribute the assimilates between the size and number of offspring. Following this approach and analysing a dataset of maize, Sadras (2007) hypothesized that seed number is highly responsive to availability of plant C assimilates during flowering, while only a narrow range of variability of seed weight was found and it can be considered constant. This hypothesis has been confirmed for several crops such as wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), narrow-leafed lupin (*Lupinus angustifolius* L.) and pea (*Pisum sativum* L.) by manipulating the source/sink ratio at flowering (Arisnabarreta and Miralles, 2008; Fischer, 1985; Sandaña and Calderini, 2012), and consciously or subconsciously assumed by physiologist for oilseed rape.

The biosynthesis of oil and proteins takes place during the seed filling period (Diepenbrock and Geisler, 1979), therefore, variations in the rate and duration of seed filling may affect seed weight, but also quality traits such as oil and protein concentration in the seed. This has been observed in maize (*Zea mays* L.), where a decrease in seed weight in response to reductions of source availability during flowering, caused an increase in oil and protein concentration (Jia et al., 2011).

It is assumed that plastic response of plant traits can be lowered by a resource limitation (Valladares et al., 2007), but the response of seed number, seed weight and quality traits to source limitation at flowering have been scarcely assessed in oilseed rape (Tayo and Morgan, 1979). Evaluating the sensitivity of seed yield components and quality traits to source limitation during flowering, can provide important information to understand the physiological factors limiting seed, oil and protein yield and to identify potential breeding targets for improving seed yield, without compromising seed quality.

1.2.4. Plasticity of seed yield components in response to plant density, N supply and source/sink ratio manipulations

The seed yield in oilseed rape, is determined by the number of plants per unit area, number of branches per plant, number of siliques per branch, number of seeds per silique and average seed weight (Diepenbrock, 2000), and the plastic responses of all of these seed yield components to environmental factors (Arnold et al., 2019). When evaluating a broad range of genotypes of legumes and cereals across several environments, it was found that seed yield is more closely related to seed number than to seed weight (Borras et al., 2004; Pereira et al., 1999; Sadras, 2007). Bradshaw (1965) used the term “hierarchy” to contrast the high plasticity of seed number against the stability of seed weight. Taking this hypothesis of the plasticity of seed number and the stability of seed weight as proven, and considering other yield components, Sadras and Slafer (2012) performed a meta-analysis in cereal crops, where the seed yield components were ranked according to their sensitivity to environmental factors. In this analysis, the number of tillers per plant was the most plastic trait, followed by the number of inflorescences and the number of seeds per inflorescence, while average seed weight was the most conservative trait.

Some studies in oilseed rape have evaluated the sensitivity of these yield components to N supply and plant population density, observing a high variation in the number of siliques per plant and in the number of seeds per silique, while the average seed weight was very stable (Habekotté, 1993; Krček et al., 2019; Kuai et al., 2016). But there are situations where seed weight becomes an important source of variation in seed yield, namely: when source/sink relations were manipulated, for example through defoliation, plant thinning, seed removal or shading during the reproductive stage (Acreche and Slafer, 2006; Borras et al., 2004; Calderini and Reynolds, 2000; Sandaña and Calderini, 2012). It has been demonstrated in oilseed rape that plant carbon status at flowering is an important determinant of the number of siliques per plant and yield per silique (Kirkegaard et al., 2018; Wang et al., 2016). Since at this stage, there is an overlap in the development of flowers, growth of siliques and setting of seeds, as well as growth of stems, leaves and branches (Iglesias and Miralles, 2014), there is a strong competition for sources among many different sinks, most of them being important seed yield components.

The evaluation of the stability of yield components to source/sink ratio manipulations under different scenarios of N availabilities and plant densities requires an analytical approach integrating plant organ growth, its response to environmental factors, and a final upscaling to biological levels relevant for agricultural research. This can be done by combining experimental and modelling approaches (Evers and Marcelis, 2019; Muller and Martre, 2019), which can contribute to foresee and quantify the potential impact of manipulated plant traits or the impact of innovative agronomical practices on seed yield.

1.2.5. Functional-structural plant modelling: an analytical tool for quantitative analysis

The need to integrate the ever-expanding body of knowledge in the plant sciences has led to the development of sophisticated modelling tools (Evers et al., 2018; Muller and Martre, 2019). Plant and crop growth models are two modelling approaches that allow the synthesis and integration of research knowledge in many areas, including efficiency in use of resources, being a valuable tool to understand and infer growth and development of different crops (Evers and Marcelis, 2019; Muller and Martre, 2019).

Crop growth models designed for oilseed rape such as LINTUL-BRASNAP (Habekotté, 1997), CERES-RAPE (Gabrielle et al., 1998), CECOL (Husson et al., 1998), APSIM-CANOLA (Robertson and Lilley, 2016), or the recently HUME-OSR (Böttcher et al., 2020), are denominated "process-based models", because they simulate physiological processes based on radiation interception (IR) and radiation-use efficiency (RUE), and capture most of the main processes governing crop productivity. However, this type of models has at least three main draw-backs: i) they allow to analyse differences in canopy pattern only in the vertical direction, making it impossible to represent the three-dimensional spatial heterogeneity that characterizes real plant canopies, ii) they focus on simulating crop performance per unit of area, but crop performance is greatly determined by growth of individual plants, particularly when increasing intra-specific competition for local resources (such as light, nitrogen or water) and conditions (such as temperature and humidity), and iii) they cannot explicitly account for plant plasticity in growth and functioning at the organ level in relation to local conditions, because they do not describe the plant structure (Evers et al., 2019). The research questions proposed in this study require an analytical tool to analyse dynamic feedbacks among organs, their source and sink dynamics at plant level, to finally upscale these processes from organ to plant and plant population, features not considered by the traditional process-based models.

Functional-Structural plant (FSP) modelling explicitly describes the development over time of the three-dimensional (3D) architecture of individual organs (Fig. 1.2a), plant (Fig 1.2b), and plant stand (Fig 1.2c) as governed by physiological processes that, in turn, depend on environmental factors (Evers and Marcelis, 2019; Vos et al., 2010). FSP modelling is a tool for exploring plant-to-plant dynamic interactions in response to light intensity (Zhu et al., 2015), environmental signals (Bongers et al., 2014), and biotic factors (de Vries et al., 2017). One of the pioneer FSP models for oilseed rape was designed and used to characterize the relationship between source-sink and plant architecture during the reproductive stage (Jullien et al., 2011). Other interesting approaches were done using CN-Wheat (Barillot et al., 2016) and NEMA (Bertheloot et al., 2011), FSP models that account for interactions between N and N dynamics at plant level but were developed to answer research questions in wheat.

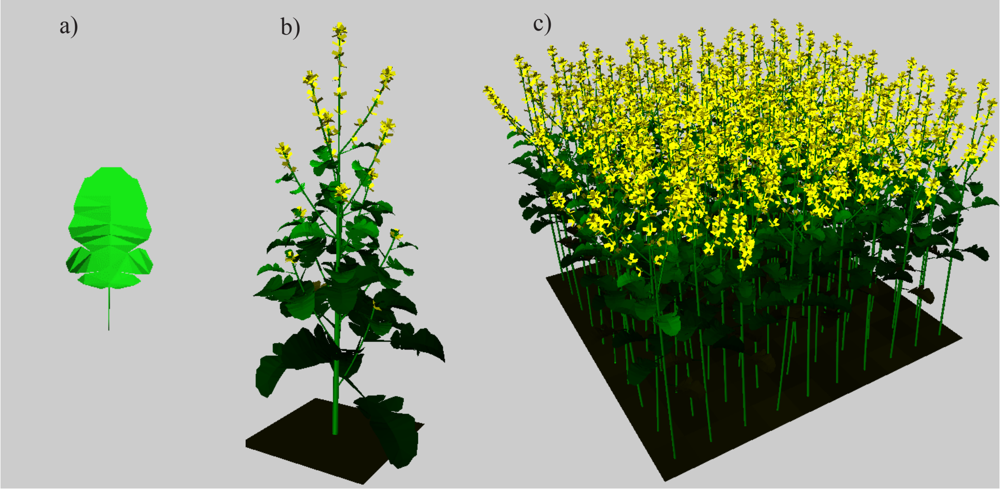

Fig. 1.2 Visualization of a) single leaf, b) plant and b) stand of plants of oilseed rape at flowering in a grid of 10 ×10,, simulated by the FSP model used in the current thesis.

Developing an FSP model for oilseed rape, considering the main architectural, phenological and physiological characteristics of this crop, will contribute to answer most of the research questions of this thesis. Thus, by combining experimental and FSP modelling approaches, it will be possible to evaluate the impact of N, plant population density and light on source-sink dynamics, plant architecture and yield formation, generating and integrating quantitative information at biological organizational levels relevant for agricultural research.

1.3. Goals and outline of this study

The overall objective of this thesis was to improve the understanding of the phenotypic plasticity of oilseed rape in response to environmental factors and cropping practices by evaluating the main drivers and mechanisms underlying plant C and N economies, its impact on stem branching pattern and potential implications on the stability of seed yield components.

For this purpose, the following research questions were addressed:
Q1. To what extent does the interaction between N supply and plant population density alter N distribution and leaf traits in oilseed rape?
Q2: What is the importance of N fertilizer supply in branch and siliques outgrowth?
Q3: Can seed weight compensate for reductions in seed number in response to artificially induced source limitation at flowering?

Q4: Do the seed yield components of oilseed rape differ in their plastic response to N supply, plant density and plant C status at flowering?

Chapter 2 explores the regulatory mechanisms underlying plant N and C economies of leaves positioned at different plant height, elucidating the extent to which the interaction between N supply and plant population density alters N distribution, and whether this interaction affects plant investment in leaf area or leaf mass per area.

In **Chapter 3** the role of N supply on plant C status and its potential effects on branch outgrowth are evaluated by combining experimental and modelling approaches. For this purpose, an FSP model is adapted to oilseed rape, using the dataset originated in the experiment described in Chapter 2. Based on plant C status approach, silique outgrowth is simulated and interactions between number of branches per plant and silique outgrowth are evaluated. Additionally, the model is used to explore the hypothetical limits of oilseed rape to produce siliques under a simulated branch inhibition. The number of siliques produced by plants with only one branch is compared with a plant with multiple branches, and the importance of branching for silique production is discussed.

Chapter 4 challenges the paradigm that assumes that seed number is the main source of variation of seed yield in response to variations in plant C status at flowering, while seed weight is a conserved trait. For this objective, the plastic response of seed number, seed weight and quality traits are evaluated in oilseed rape by performing a source limitation at flowering. The extent to which plant C status determines the plastic responses of seed number and seed weight, the impact on oil and protein content are here discussed integrating concepts analysed in Chapters 2 and 3, while a potential breeding target is proposed.

Chapter 5 builds on Chapter 4 and quantifies the sensitivity of the seed yield components of oilseed rape to different environmental conditions. For this purpose, a dataset collected from experiments under contrasting N supplies and plant populations and from experiments of source-limitations at flowering is evaluated. A ranking of plastic responses among yield components is obtained, and the impact of different environmental factors on these results is analysed. This chapter goes deep into the effects of source/sink ratio manipulations during the reproductive stage on the seed yield components. For this purpose, source availability and sink strength are modified at flowering, using the FSP modelling approach described in Chapter 3. The importance of plant C status as determinant of seed yield, and the contribution of the FSP modelling approach as a tool to elucidate research questions related with trait plasticity are discussed.

Finally, **Chapter 6** summarises the main findings of this study and discusses potential applications for agronomic and breeding purposes, while remaining knowledge gaps are identified.

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Chapter 2

To what extent does the interaction between nitrogen supply and plant population density determine nitrogen distribution and leaf traits in oilseed rape plants?

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Abstract

Understanding the regulatory mechanisms underlying plant nitrogen (N) and carbon (C) economies, and their role in defining phenotypic plasticity in oilseed rape, is necessary for designing new strategies to optimize plant and canopy C assimilation to improve potential yield of oilseed rape. This paper aims to elucidate the extent to which the interaction between N supply and plant population density alters N distribution in oilseed rape plant (*Brassica napus* L.), and whether this interaction changes plant investment in leaf area or leaf mass per area.

Spring oilseed rape was grown at two rates of N supply (50 and 150 kg ha^{-1}) and two plant population densities (50 and 150 plants m^{-2}). Photosynthesis, leaf area, biomass and N content of selected leaves were measured 7 days before flowering. The interaction between N supply and plant population density altered significantly N per unit of area, which is the main determinant of photosynthesis. This interaction also affected leaf mass per unit area, while N supply determined N content per unit leaf mass. These results suggest that the interaction between N supply and population density significantly affects both nitrogen distribution and leaf mass per area, which together with effects on plant height, could have important implications for light distribution and therefore for C assimilation at plant level.

Additional keywords: *Brassica napus*, leaf area, leaf mass per area, light interception, nitrogen distribution, nitrogen supply

2.1. Introduction

During the last 50 years, oil crops have been the commodities with the greatest relative increase in the contribution to the world's food supply (Khoury et al., 2014). Oil from oilseed rape (*Brassica napus* L.) is the third most consumed vegetable oil after soybean and palm oil, while it is one of the healthiest edible oils for human consumption (Momoh et al., 2004). Additionally, the rising demand of biofuels (Rondanini et al., 2012) has resulted in an exponential growth of oilseed rape production since 1980, reaching 71 million tonnes in 2017 (FAOSTAT, 2020). Future increases in crop production should be accompanied by improved sustainability of crop production systems; therefore, there is a need for developing and improving crop nitrogen (N) management which can maintain high levels of production, while minimizing N input (Dawson et al., 2008), and more insight in regulatory mechanisms controlling plant N economy is vital for improving N-use efficiency (Hirel et al., 2007; Stahl et al., 2019). Since N economy is closely associated with carbon (C) economy (Gastal et al., 2015) interaction between both should be considered.

Oilseed rape has shown wide compensation among yield components across a wide range of plant population densities (Roques and Berry, 2016; Seepaul et al., 2016), opening the opportunity to reduce sowing rates. At low plant densities, yield compensation is reached with higher biomass per plant and, lower biomass per square meter than at conventional sowing rates. As canopy architecture and the light environment at each given plant phytomer is modified by plant population density, the plant acclimation and photosynthetic performance would be different as well. However, little is known on the effects of the interaction between N supply and light interception on plant and leaf architectural traits.

Leaf photosynthetic capacity is strongly related to N content per unit of leaf area (N_{area}) (Hikosaka, 2004), but wide variation in the photosynthetic capacity for a given N_{area} has been observed in different plant species (Bloomfield et al., 2014; Evans, 1989). N_{area} is associated with leaf mass per unit of leaf area (*LMA* mg mass cm^{-2}), which is the ratio of dry mass per unit of leaf area and therefore, an indicator of the plant investment in light-capturing and photosynthetic organs (Lambers and Poorter, 1992). This contribute to explain the sensitivity of *LMA* to different plant population densities observed (Gan et al., 2002). The response of *LMA* to N supply is more variable: this trait has shown to be negatively correlated with N supply (Gammelvind et al., 1996), or not correlated at all (DeJong et al., 1989). The other physiological determinant of N_{area} is N content per leaf mass (N_{mass}), but the environmental factors affecting N_{mass} are poorly understood, showing variable responses to light interception (Osada, 2013) and N supply (Ordonez et al., 2009; Rosati et al., 2000). The effects of both N supply and plant population density on LAI or LMA have been evaluated in maize (*Zea mays* L.), but no significant effects were observed (Amanullah et al., 2007). From the results of this last study, is possible to observe an interaction between N supply and plant population density, but this was not considered in their analysis.

The N demand of plants is not completely matched by the N supplied as fertilizer; available N is variable at short time scale due to environmental constraints that affect the N cycle (Stenger et al., 2002). When N is in short supply, N distribution in plants has shown to be more dependent on N soil status and subsequent N uptake by the plant, rather than on leaf light interception (Werger and Hirose, 1991). *Ipomoea tricolor* (Hikosaka et al., 1994), grown at low N rate, showed a decrease in N_{area} associated with ageing of leaves, while leaf N_{mass} of *Xanthium canadense* was strongly affected by leaf position on the plant, independent of the relative photon flux density in experiments under low N availability (Anten et al., 1998).

A substantial body of literature about N distribution stems from studies performed on woody, perennial or wild plant species (Anten et al., 1995; DeJong et al., 1989; Ordonez et al., 2009), which have different preferences for N forms (i.e., NO_3^-, NH_4^+), mycorrhizal associations, amounts of N storage and adaptive responses to low N levels (Aerts and Chapin, 2000); therefore their conclusions cannot be directly extrapolated to crop species such as oilseed rape, and especially when under different plant population densities.

The understanding of the ways through which the combined effect of N supply and plant population density determine N distribution, and of their impact on N and C economies is of major importance for the design of new strategies to optimize plant and canopy C assimilation to improve grain yield of oilseed rape. For that reason, the current study aims to address the following research questions: 1) to what extent does the interaction between N supply and plant population density alter N distribution in oilseed rape? 2) does the plant respond to this interaction by modifying assimilate investment in leaf area or by modifying leaf mass per unit of area?

To answer these questions, we focused at seven days before flowering because the period bracketing flowering is essential for branch appearance in oilseed rape (Al-Barzinjy et al., 2003) and seed yield determination (Dreccer et al., 2000b). Moreover, while exploring this period, N re-mobilization processes from the evaluated leaves to reproductive sinks during later stages are largely avoided.

2.2. Materials and methods

2.2.1. Experimental set up and treatments

On 17 April 2013 spring oilseed rape (*B. napus* ‘Solar CL’) was sown under an open-sided shelter with transparent roof in Wageningen, the Netherlands (51° 58′ N, 5° 40′ E). The treatments consisted of combinations of two rates of N fertilization: 50 (N50) and 150 (N150) kg ha^{-1} and two plant population densities: 50 (D50) and 150 (D150) plants m^{-2}, with the goal to obtain contrasting N supply and light environments and, thus, different N distribution at plant level. The plant population density of 150 plants m^{-2} was chosen according to similar experiments performed in spring oilseed rape under similar experimental (Dreccer et al., 2000a). On the other hand, we assessed a contrasting low population density (50 plants m^{-2}) taking into account that this density increases branching and modifies carbon partitioning on plants. While, the N supplies of 150 and 50 Kg N ha^{-1} applied in our experiment were also similar to the highest and lowest N availabilities used by Dreccer et al. (2000a).

Treatments effects were tested on plants growing in containers (0.7 × 0.9 m), which contained from bottom to top: a layer of 35 cm of sandy soil, upon which fertilizers ($Ca(H_2PO_4)_2$ and $K_2SO_4.MgSO_4$) were applied and covered. On this layer, seeds were placed in a hexagonal spatial pattern and covered with a layer of 2 cm soil.

The containers were arranged closely together to ensure canopy homogeneity, and twelve guard containers were placed around the experimental containers aimed to avoid border effects on the plants that were to be measured. As in previous experiments (Evers et al., 2006; Lyons et al., 2009; Retuerto et al., 1996), treatments were assessed in a single container and we used individual plants as pseudo-replicates (Hurlbert, 1984) and, in agreement with the homogeneity of the soil (coefficient of variation of N present in the soil at sowing was 3.74%) and plants in this well-managed experiment allowed very high similarity across plants at each experimental unit. In addition, the containers were re-arranged two times to reduce position effects in the open-sided shelter. Phenological growth stages in oilseed rape were recorded according to the BBCH phenological scale (Meier, 2001).

The N fertilizer KAS-27 (13.5% NO_3^- and 13.5% NH_4^+) was applied to the soil (containing 30 kg N ha^{-1} and 3.1% organic material) to reach 50 and 150 kg N ha^{-1}. In the lower N treatment 50 kg N ha^{-1} was applied 2 days after emergence, while in the higher N treatment 150 kg N ha^{-1} was split into two applications: 100 kg ha^{-1} 2 days after emergence (BBCH 10) and 50 kg ha^{-1} when the 5th internode was expanded (BBCH 35) (Fig. 2.1).

The containers were irrigated daily or twice per day dependent on the environmental temperature, while, fungus diseases such as Phoma lingam and Sclerotinia sclerotiorum were chemically controlled by using the fungicides prothioconazole (Proline®) and iprodione (Rovral®), respectively. Weeds were manually removed.

Phenological stage:					
BBCH 10	BBCH 15	BBCH 37	BBCH 53	BBCH 61	BBCH 62
Leaf appearance:					
(Cotyledon)	(Rosette)	(Leaf 9 appearance)	(Leaf 11 appearance)	(Leaf 9.1 appearance)	(Final evaluation)
N fertilization and measurements:					
N fertilization (1st dose)		N fertilization (2nd dose)			- Destructive sampling, and photosynthesis measurements

Fig. 2.1 Schematic diagram of oilseed rape developmental stages, leaf appearance, moment of N fertilization and measurements.

2.2.2. Temperature conditions

Temperature was recorded (Data taker DT600, Datataker Data Loggers, Cambridgeshire, UK) with shielded thermocouples (type T, TempControl Industrial Electronic Products, Voorburg, the Netherlands) at 15 min intervals from sowing to flowering. Thermocouples were placed at mid-canopy and below the soil surface at 7 cm depth. Plant development and leaf appearance were expressed in thermal time (*TT*) after emergence and calculated by summing daily mean temperatures (T_{mean}) using a base temperature (T_{base}) of 0 °C for spring oilseed rape, *n* is the variable being summed up to and *i* is the starting term (Kirkegaard et al., 2012):

$$TT = \sum_{i}^{n} (T_{\mathrm{mean\ i}} - T_{\mathrm{base}}) \qquad \text{(Eq. 2.1)}$$

For the first 15 days after emergence, thermal time was estimated based on soil temperature, since the apex was below soil surface; canopy temperature was used thereafter.

2.2.3. Plant measurements

One week after emergence, 10 homogeneous (i.e. of similar plant height, leaf size and color) and representative plants grown in the center of the evaluated containers, were randomly selected and labelled for each treatment (5 plants for destructive measurements and 5 for photosynthesis evaluation) and the leaves were coded according to the phytomer rank to which they belonged. A leaf was considered to have appeared halfway between the last observation when it was absent and the first observation when the tip was visible. For each plant selected, the time of leaf tip appearance of leaf 9 and 11 (i.e that at phytomer of rank 9 and 11 respectively) on the main stem as well as leaf 9.1 (i.e the leaf of phytomer 1 on the primary branch outgrowing from phytomer 9 on the main stem) was recorded twice a week from emergence (BBCH 10) to flowering (BBCH 62) in order to estimate the time of leaf appearance. Because in oilseed rape leaves placed at lower phytomers differ in their size, shape and presence of petiole in comparison with middle and upper leaves (Bailey and Bailey, 1976), and because the beginning of flowering assimilate remobilization and/or senescence was evidenced in leaves positioned below phytomer rank 3, only sessile leaves positioned at higher phytomer ranks were evaluated. Plant height was measured at the beginning of flowering, as the distance from the basis and the top of the main stem.

Light interception (*IPAR*) was measured twice per week at canopy level in all the containers evaluated, so light interception by leaves located in a given layer was estimated by assuming *IPAR* to attenuate through the leaf canopy following the Beer-Lambert's law (Monsi and Saeki, 2005):

$$IPAR = PAR_{\mathrm{inc}} \times (1 - e^{-k \times LAI}) \qquad \text{(Eq. 2.2)}$$

k is the light extinction coefficient and depends upon the architecture of a canopy and transmission of radiation through individual leaves (Monteith, 1973) and it was estimated by the function:

$$k = -\ln\,(PAR_{\mathrm{trans}} / PAR_{\mathrm{inc}}) \,/\, LAI \qquad \text{(Eq. 2.3)}$$

Light incident (PAR_{inc}) was recorded by the BF5 Sunshine Sensor (Delta-T Devices Ltd., Cambridge, United Kingdom), which was placed on a horizontal level 1.5 m close to the container evaluated, and light transmitted (PAR_{trans}) was measured at ground level respectively, with a 1 m sensor probe of the SunScan Canopy Analysis System (Delta-T Devices Ltd., Cambridge, United Kingdom).

Leaf area index (*LAI*) is the total leaf area per plant multiplied by the plant population density in one square meter. Leaf area of each individual leaf at each phytomer from the top of the canopy was measured. Cumulative *LAI* was estimated for each rank phytomer and in this way, *IPAR* of individual leaf was estimated by Eq. 2.2, assuming that each leaf layer has a similar *k*.

Net assimilation rate (A_n) measurements were done 5 - 7 days before flowering on leaves 9, 9.1 and 11 for treatment N150-D50 and on leaves 9 and 11 for the treatments N50-D50, N150-D150, N50-D150 because these treatments did not produce branches nor leaf 9.1. At that moment, the evaluated leaves were fully expanded. The photosynthesis rate was determined with a LI-COR 6400XT Portable Photosynthesis System (LI-COR, Lincoln, Nebraska, USA) at saturated incident light on surface area (I_{inc}) (I_{inc}=1500 µmol m^{-2} s^{-1}; CO_2=390 µmol mol^{-1}) and at a leaf temperature of 25 °C.

Seven days before flowering (BBCH 62) a destructive sampling was performed. Five plants per treatment (previously tagged) were harvested and leaf area of individual leaves 9, 9.1 and 11 was assessed (LI 3100; LI-COR, Lincoln, Nebraska, USA). After oven drying (at 70 ºC during 48 hours) dry matter of leaves was also recorded. The remaining leaves and stems were also collected, to estimate dry matter and N uptake, but detached leaves were not considered for the analysis. N contents (%) of individual leaves, remaining leaves and stems were determined in an N elemental analyser based on the combustion method of Dumas (Flash 2000, Thermo Fisher Scientific Inc., Delft, the Netherlands).

With this information, N_{area}, LMA, N_{mass} and N uptake were calculated (see Table 2.1 for an explanation of all abbreviations used). Leaf N content per unit of leaf area (N_{area}), of leaves 9, 9.1 and 11 was determined as:

$$N_{\mathrm{area}} = LMA \times N_{\mathrm{mass}} \qquad \text{(Eq. 2.4)}$$

where *LMA* is the leaf mass per unit of area, and N_{mass} is the amount of leaf N per unit of leaf dry weight.

Table 2.1 List of abbreviations, their definitions and units.

Symbol	Variable name	Definition	Units
A_n	Rate of photosynthesis	Net CO_2 uptake per leaf area per time	µmol CO_2 m^{-2} s^{-1}
IPAR	PAR intercepted	PAR intercepted by the canopy	µmol m^{-2} s^{-1}
k	Light extinction coefficient	Extinction coefficient for light in a stand	-
LAI	Leaf area index	Leaf area / ground area	m^2 m^{-2}
LMA	Leaf mass per unit of leaf area	Leaf dry matter / leaf area	mg mass cm^{-2}
PAR	Photosynthetically active radiation	Spectral range of solar radiation from 400 to 700 nm	nm
PAR_{inc}	PAR incoming	PAR incoming above the canopy	µmol m^{-2} s^{-1}
N_{area}	N content per leaf area	LMA / N_{mass}	mg N cm^{-2}
N_{mass}	N content per unit of mass	Amount of N / leaf dry weight	mg N g^{-1} leaf
NO_3^-	Nitrate	Nitrate content	g m^{-2}
N_{total}	Total mineral nitrogen	$NO_3^- + NH_4^+$	g m^{-2}
N uptake	Nitrogen uptake	N contained in the plant	g $plant^{-1}$
TT	Thermal time	Accumulation of daily mean temperature above a base temperature	°C day

2.2.4. Soil sampling

Nitrogen is present in the soil as a mixture of organic and inorganic forms (Miller and Cramer, 2005), from which nitrate (NO_3^-) is the most absorbed form of N (Arkoun et al., 2012), and the one most associated to developmental and growing processes in oilseed rape (Laine et al., 1995; Li et al., 2013). In addition, the status of this nutrient varies over time; for that reason, the time course of NO_3^- availability is a more accurate way of analysing effects of N supply on oilseed rape than total mineral N availability. For this purpose, soil samples were collected at four different stages between sowing and beginning of flowering (BBCH 62) and total mineral N ($NO_3^- + NH_4^+$) and nitrate were determined based on the extraction with the 0.01 M Calcium Chloride method (Houba et al., 2000).

To obtain the dynamic of N availability and then, the NO_3^- available at the moment that each leaf appeared, a 3-parameter power function was fitted to the NO_3 measured:

$$Y = A + B \times R^{TT} \qquad \text{(Eq. 2.5)}$$

where Y is NO_3^- availability, A is the intercept, B determines the slope of the curve, R is the asymptotic value and TT is thermal time.

2.2.5. Statistical analysis

Experimental data were subjected to analysis of variance using GENSTAT 17th edition (Payne et al., 2011) and differences were considered to be statistically significant at a probability level of 5%. To evaluate the effects of N supply, plant population density and their interaction at plant level (i.e., plant height, N uptake per plant), a two-way ANOVA was used and when significant effects were found, differences between high vs low N level or between high and low plant population level were evaluated by means of a two-sample t-test. When leaf position was also included as a factor (i.e. leaf appearance, leaf area, N content per leaf, N_{area}, LMA, N_{mass}) evaluated variables (Y_{ijk}) were analysed by using the following model:

$$Y_{\mathrm{ijk}} = \mu + N_{\mathrm{i}} + D_{\mathrm{j}} + ND_{\mathrm{ij}} + NDL_{\mathrm{ij(k)}} + \varepsilon_{\mathrm{ijk}} \qquad \text{(Eq. 2.6)}$$

where μ represents the mean of the observations and the effects of the treatments are represented as follows: N_{i} is the fixed effect of N supply, D_{j} is the fixed effect of plant population density,

ND_{ij} represents the interaction between N supply and plant population density, NDL_{ijk} represents the effect of leaf position within N supply and population density, plus a residual ε_{ijk}, which represents the random variation. Since not all the treatments produced leaf 9.1, the effect of leaf position (L_k) was not evaluated. When significant effects were found, differences among treatments were evaluated by means of the Fischer LSD test.

Linear and exponential models were used to assess the associations between variables and adjusted r-square (*adjusted* r^2) was used to evaluate goodness of fit of these models, which is the difference between residual and total mean squares of the total mean square. The adjustment takes account of the number of parameters in the model compared to the number of observations (Payne et al., 2011).

2.3. Results

2.3.1. Plant and leaf characteristics

The timing of emergence (BBCH 10), rosette formation (BBCH 25), and beginning of flowering (BBCH 62) were not affected ($P>0.05$) by the treatments. Because there was no interaction between N supply and plant population density on plant height ($P>0.05$) their effects on this trait were analysed independently. Plants of treatment N150 were 12 cm taller than plants grown under N50, while plants grown at low plant population density (D50) were 15 cm taller than those at high density (D150) (Fig. 2.2a). Plants in all treatments had 13 to 14 phytomers on the main stem.

Remarkable differences in leaf area at phytomer ranks in response to N supply, and to a lesser degree to plant population density, were observed (Fig. 2.2b), while yellowing and senescence were evident from phytomer 3 downwards. In all the treatments, leaves positioned on phytomers 1 and 2 had already dropped at that time. The area of leaves positioned in phytomer ranks 3 and 4 was slightly higher in N150-D150 than in N150-D50, while these differences disappeared at higher phytomers ranks. This means that at early stages, when plant competition was less of a limiting factor, plants grown at higher densities expanded leaves slightly larger than at low plant population density.

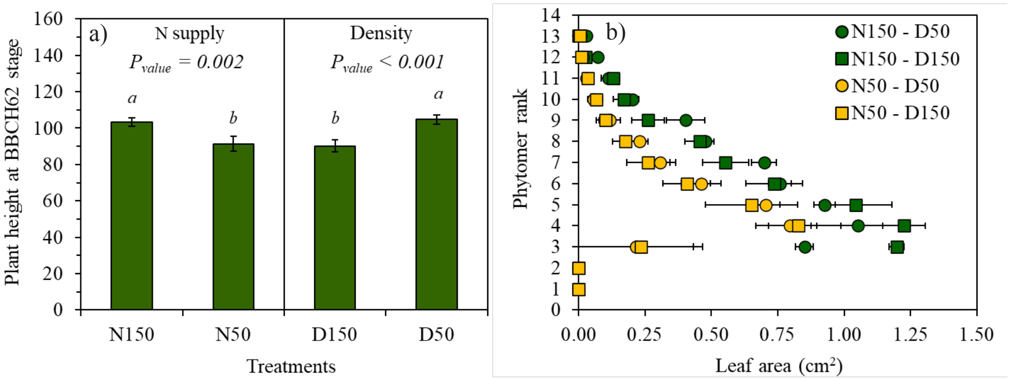

Fig. 2.2. a) Plant height in response to N supply and plant population density. Different letters indicate significant differences across treatments (P<0.05) and vertical bars indicate standard error of means, and b) leaf area for each phytomer rank (includes main stem and branch leaf area) at seven days before flowering (BBCH 62) in response to N supply and plant population density. Horizontal bars indicate standard error of means at each phytomer rank.

A significant interaction among N supply, plant population density and leaf position was observed in leaf appearance, individual leaf area and N content per leaf of the evaluated leaves (Table 2.2). Among the three positions measured, the leaf positioned at phytomer 9 was the first to appear, followed by leaves 11 and 9.1 in second and third place, respectively. While the timing of appearance was not affected in leaf 9 by the treatments and their interaction, leaf 11 appeared earlier in treatments N50-D150 and N50-D50 than in treatments N150-D150 and N150-D50. Across treatments, leaf area of leaves 9 and 11 was larger in N150-D50 than in treatments with low N supply (N50-D50 and N50-D150), and leaf 9.1 was not different from the leaf area of leaf 11 (P>0.05). Total N content of leaf 9 and 11 was higher in N150-D50 than in the other treatments.

2.3.2. *Leaf N content (N_{area}) and photosynthesis*

There was a significant interaction effect (P<0.05) between N supply and plant population density on N_{area} in leaves 9, 9.1 and 11 (Fig. 2.3a). The highest N_{area} value was reached in leaves 9 and 11 (0.16 and 0.20 mg cm^{-2} respectively) grown under N150-D50, while leaf rank 9.1, the youngest leaf, recorded the lowest N_{area} (0.10 mg cm^{-2}). N_{area} of leaves 9 and 11 grown under N150-D150, N50-D50 and N50-D150 did not differ significantly (P>0.05). Light saturated photosynthesis rate per area (A_{n}) was asymptotically related to N_{area} across treatments and leaf positions (Fig. 2.3b), whereas at low N_{area}, A_{n} increased markedly until 0.12 mg N cm^{-2} reaching a plateau at around 0.20 mg N cm^{-2}. This trend in oilseed rape has also been observed in a wide diversity of other crops including sunflower (*Helianthus annuus*), kenaf (*Hibiscus cannabinus*), and cynara (*Cynara cardunculus*) (Archontoulis et al., 2012; Trapani and Hall, 1996)

2.3.3. Physiological and structural determinants of N_{area}

LMA was affected (P=0.003) by the interaction between N supply, plant population density and leaf position. Within a treatment, differences in *LMA* (Fig. 2.4a) among the evaluated leaves were significant (P<0.05) in N150-D50 where leaves 9 and 11 showed higher *LMA* than leaf 9.1, while the other treatments did not show differences among leaves. Among treatments, *LMA* of leaf 11 was higher in treatments N150-D50 and N50-D50 than in N150-D150 and N50-D150. On the other hand, N_{mass}, the other physiological determinant of N_{area}, was analysed considering both N and plant population density as independent factors because there was no interaction between them (Fig. 2.4b). High N supply (N150) increased N_{mass} by 65% compared with the value for plants grown at low N supply (N50), while the high plant population density (D150) showed N_{mass} 11% lower than the low density (D50).

Table 2.2. Time of appearance, leaf area and N content of leaves at phytomers 9, 9.1 and 11.

Treatment	Leaf position	Leaf appearance (°C day)	Individual leaf area (cm^2 per leaf)	N content per leaf (mg N per leaf)
N150-D50	Leaf 9.1	690.9 e	11.83 de	1.180 cd
	Leaf 11	539.1 d	14.86 cd	2.868 b
	Leaf 9	473.1 a	60.74 a	9.916 a
N150-D150	Leaf 11	535.2 cd	9.44 def	0.892 cd
	Leaf 9	470.4 a	19.26 c	2.184 bc
N50-D50	Leaf 11	527.6 bc	6.27 ef	0.756 d
	Leaf 9	466.9 a	26.59 b	2.729 b
N50-D150	Leaf 11	523.7 b	3.44 f	0.136 d
	Leaf 9	469.3 a	9.19 def	0.722 d
	N supply (N)	***	***	***
	Plant population density (D)	***	***	***
	N × D × Leaf position	***	***	***

Different letters indicate significant differences between leaf positions in response to treatments according to ANOVA and Fischer LSD test, while asterisk indicates differences between treatments (P < 0.001).

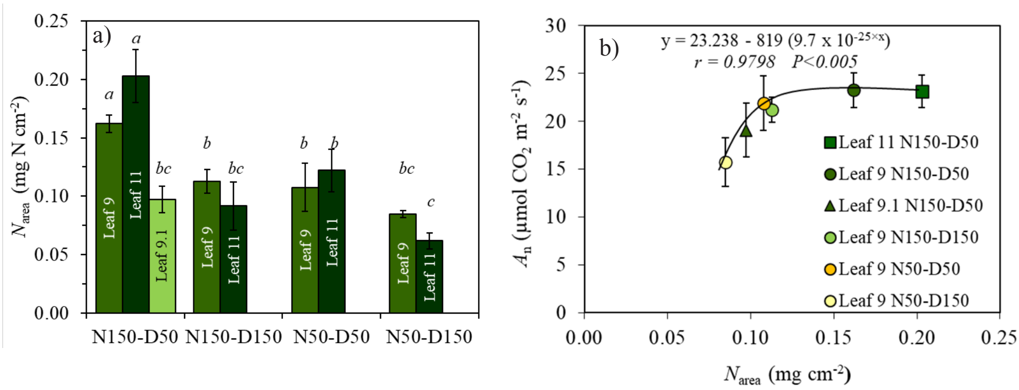

Fig. 2.3. a) Leaf N content per area (N_{area}) of leaves at different phytomer ranks in response to N supply and plant density; and b) light saturated photosynthesis (A_n) (I_{inc}=1500 µmol m^{-2} s^{-1}; CO_2=390 µmol mol^{-1}) in relation to N_{area} at seven days before flowering (BBCH 62). Different letters indicate significant differences across treatments ($P<0.05$) and vertical bars indicate standard error of means.

N_{area} was closer associated with *LMA* (Fig. 2.5a) than with N_{mass} (Fig. 2.5b) and showed a linear relationship depending on N supply. The intercepts of those relationships were lower at N150 than at N50, therefore the minimum N_{area} was reached with smaller *LMA* at N150 than at N50. A positive relationship was observed between *LMA* and light interception by leaves located in a given layer for treatments grown at low plant population density (N150-D50 and N50-D50) irrespective of N supply (Fig. 2.6) ($P<0.05$). Thus, LMA was more sensitive to plant population density than to N supply. However, this trend was not valid for the high-density treatments (N150-D150 and N50-D150).

At increasing intraspecific competition, other environmental factors become limiting for leaf structure (i.e., red: far-red ratio, belowground competition). To assess the efficiency of the N treatments, N uptake per square meter was evaluated (Fig. 2.7a), and N uptake per square meter of N150 was two times higher than that of N50 ($P<0.01$), while the interaction between N supply and plant population density was not significant ($P>0.05$). At plant level (Fig. 2.7b), the interaction between N supply and plant population density was significant ($P<0.01$) and treatment N150-D50 showed the highest ($P<0.05$) N uptake per plant with 0.29 g N per plant followed by N150-D150 and N50-D50, while N50-D150 recorded the minimum N uptake with only 0.04 g N per plant.

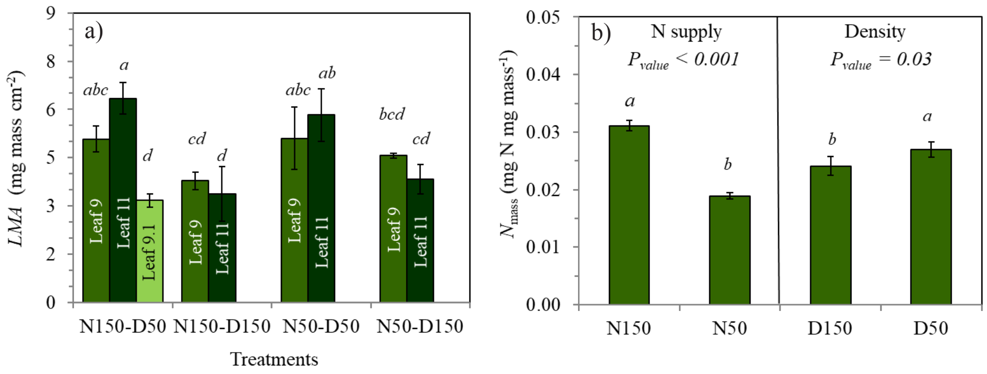

Fig. 2.4. a) Leaf mass per unit of area (LMA) and b) N content per unit of mass (N_{mass}), of leaves at different phytomer ranks in response to N supply and plant population density at seven days before flowering (BBCH 62). Different letters indicate significant differences across treatments ($P<0.05$) and vertical bars indicate standard error of means.

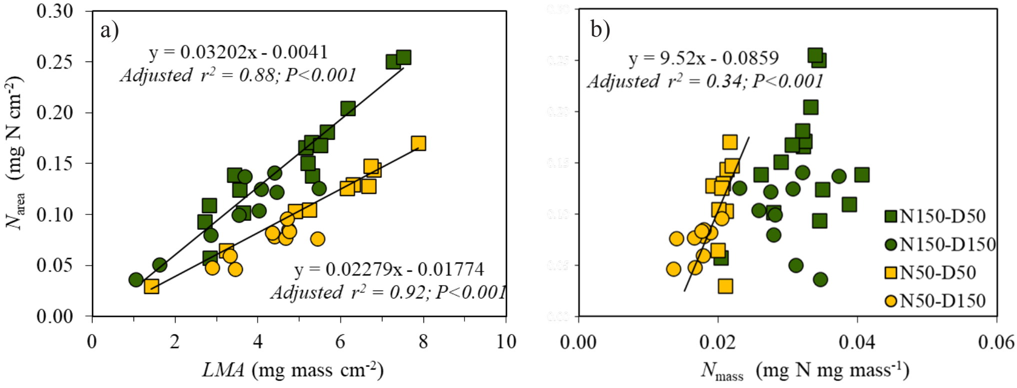

Fig. 2.5. Leaf N content per unit of area (N_{area}) versus a) leaf mass per unit of area (LMA) and b) N content per unit of mass (N_{mass}) at seven days before flowering (BBCH 62). Each figure corresponds to an individual leaf.

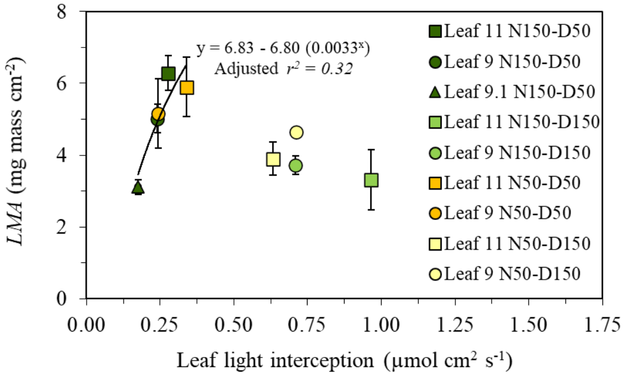

Fig. 2.6. Relationship between leaf mass per unit of area (LMA) and light interception by leaves located in a given layer at seven days before flowering (BBCH 62). Vertical bars indicate standard error of means.

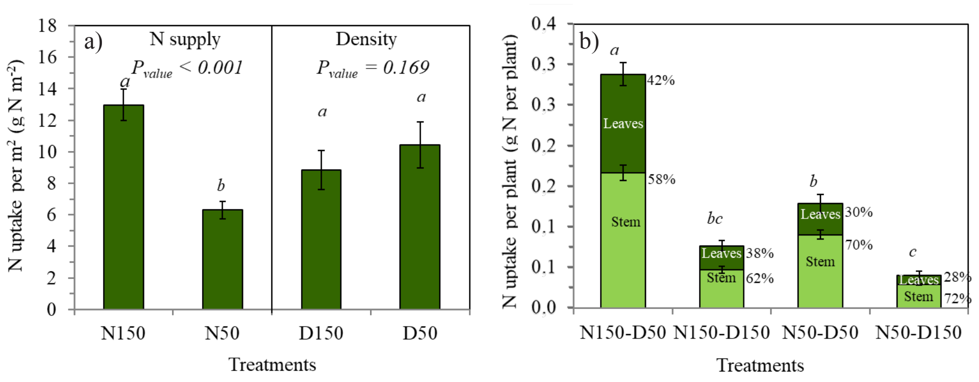

Fig. 2.7. a) N uptake per square meter, and b) N uptake per plant and percentage of N in leaves and stem in response to N supply and plant population density at seven days before flowering (BBCH 62). Different letters indicate significant differences across treatments (P< 0.05) and vertical bars indicate standard error of means

Nitrogen partitioned to leaves in N150-D50 and N150-D150 was almost 10% higher than in N50-D50 and N50-D150, confirming that plants grown under N150 accumulated and partitioned more N to the leaves than plants grown under N50, since the latter grew under more restrictive N conditions and partitioned more N to the stem.

NO_3^- availability per plant decreased over time across the treatments, reaching its minimum level at around flowering (Fig. 2.8a). From the fitted function (Eq. 5) and the values of leaf appearance (Table 2.2), NO_3^- available per plant at the moment the leaves evaluated appeared was calculated. An asymptotic trend between N_{mass} and NO_3^- availability at the time of leaf appearance was observed (Fig. 2.8b), while *LMA* was not associated with NO_3^- availability (data not shown).

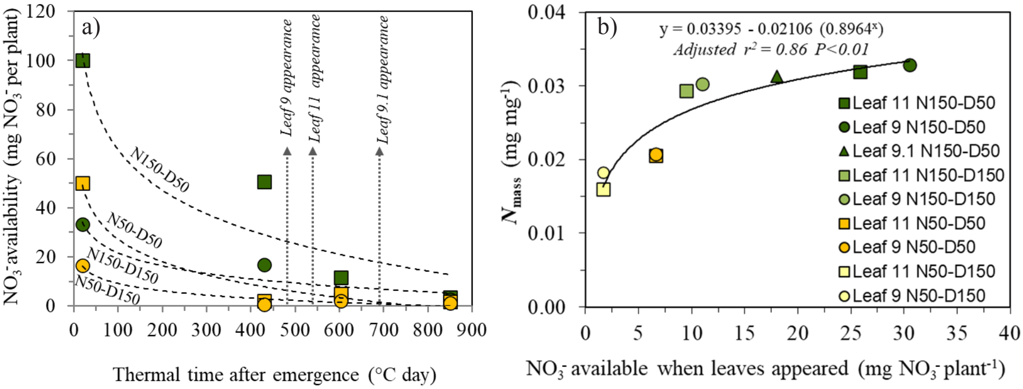

Fig. 2.8. a) Nitrate (NO_3^-) availability as a function of thermal time since emergence until flowering (vertical arrows represent the moment when leaves evaluated appeared). Fitted function were:

N150-D50, $y = 3.60 + 103.90\ (0.997^x)$, ($r = 0.9182$);

N150-D150, $y = 1.41 + 34.27\ (0.9973^x)$, ($r = 0.9407$);

N50-D50, $y = 6.60 + 91.24\ (0.962^x)$, ($r = 0.8695$);

N50-D150, $y = 1.68 + 31.50\ (0.9620^x)$, ($r = 0.9813$); and

b) relationship between N_{mass} and NO_3^- availability at the moment when leaves 9, 9.1 and 11 appeared.

2.4. Discussion

2.4.1. Effects at plant level

Both assessed factors, N supply and plant population density, affected plant growth and architecture, while plant phenology was not affected by the treatments. Only the interaction among N supply, plant population density and leaf position, affected leaf appearance of the evaluated leaves. Low N supply reduced plant height and leaf area, while at low density plants were taller but developed smaller leaves than at high density. Reductions of plant height and individual leaf area in response to low N supply corroborate previous work in *Triticum aestivum* (Reddy and Prasad, 1979), *Zea mays* (Yin et al., 2012) and *Gossypium hirsutum* (Muharam et al., 2014). According to the literature (Kuai et al., 2016) the interaction between N supply and plant population density influences the leaf area index, which determines the proportion of light intercepted by the canopy. It has been observed that the higher impact of N supply on leaf area is at early stages (i.e. budding stage), while the effect of plant population density is at later stages (i.e., seed filling stages) (Kuai et al., 2016). As our study focused on the phenological stage of beginning of flowering (BBCH 62), both effects resulted as an emergent property of the interaction as a consequence of the effective availability of resources per plant.

On the other hand, an increase in plant density induces competition for solar radiation and nutrients among individuals, leading to higher plant height and thinner stems, resulting in an increase in lodging risk (Xue et al., 2016), one of the most important problems in farming systems. In our study and at high N rates, the highest plant height was found at low plant density (150 plants m^{-2}), and not at high plant density (50 plants m^{-2}). These results are supported by studies performed in oilseed rape in field experiments (when comparing sowing rates of 15 vs 45 plants m^{-2}) (Li et al., 2014) and flax (*Linum usitatissimum*) (Lafond, 1993), were increasing population density higher decreased plant height. This behaviour is the resultant of an intensive intra-specific competition for resources (i.e., light), at developmental stages when the requirements are higher than the availability of resources, as has been demonstrated in sunflower (Xiao et al., 2006) and now in oilseed rape. Thus, at conventional plant densities, increasing plant population density leads to higher plant height, but there is a critical level above which further increases in plant population density will cause a reduction in plant height, due to a shortage of assimilates available for plant growth. Variations in leaf area in response to N supply were remarkable in all the evaluated leaves, while a delay in leaf appearance was evident only in the final leaves evaluated (i.e., leaves 11 and 9.1).

Under optimal conditions, N available in the soil is enough to satisfy the plant N demand (Gastal et al., 2015). Thus, N is absorbed, assimilated and distributed in the plant to maximize photosynthesis (Monsi and Saeki, 2005) by means of building metabolic (photosynthesis and meristematic activity) and structural tissues necessary for plant architecture (leaves, stems) (Gastal et al., 2015). But, when plant N demand exceeds N availability, N and C are mostly allocated to the roots compartment and shoot/root and leaves/stem ratios are reduced (Gastal et al., 2015), thus reductions in leaf area and delays in leaf appearance are expected. Although our study did not include measurements of root biomass, the lower N uptake per square meter (Fig. 2.7a) and the lower N leaves/stem ratio (Fig. 2.7b) in treatments with low N supply, added to the later appearance of the younger leaves (leaves 11 and 9.1) coincided with the moment when N availability most likely started to become a limiting factor (Fig. 2.8), confirming that N availability played an important role in leaf development and expansion. Since detached leaves were not considered as part of the evaluation, N uptake and N redistribution were possibly slightly underestimated.

2.4.2. Effects at leaf level

Leaf traits like leaf thickness, palisade depth and mesophyll cell density are summarized in a single concept: *LMA* (Thompson et al., 1992). The *LMA* showed a significant response to the interaction among N supply, plant population density and leaf position. These results differ to studies performed crops like soybean (Gan et al., 2002) or in trees like *Picea abies* (Niinemets, 1997), in which *LMA* is mainly modified as a result of leaf light conditions, while inter-specific studies (Poorter et al., 2009) show moderate responses of *LMA* to nutrient shortage. The same response was observed in *Prunus persica* (DeJong et al., 1989), whereas by contrast Jullien et al. (2009) observed in winter oilseed rape an increase of *LMA* grown at 200 kg ha^{-1} of N supply in comparison with higher fertilization rate (300 kg ha^{-1}). Discrepancies among these studies could be due to the fact that effects of N and light are hidden by their interaction. In addition, there are differences in responses of *LMA* to N supply among plant species and genotypes (Jullien et al., 2009; Poorter et al., 2009). Furthermore, assimilates allocation to different organs is a dynamic process that varies among phenological stages (Gastal et al., 2015). For that reason, differences in *LMA* observed in spring oilseed rape during a specific phenological stage (BBCH 62) are not totally comparable with studies performed in winter oilseed rape at another phenological stage (Jullien et al., 2009).

It is known that N supply has higher impact on IPAR and LAI at early stages (i.e., budding stage), while density has the most significant impact during the seedling stage. Thus, the interaction between N supply and plant population density influences the IPAR and LAI during early and mid (i.e., seedling, budding and blossom) developmental stages (Kuai et al., 2016). The other determinant of N_{area} is N_{mass}, which was affected more by N supply than by plant population density. This is supported by a global study of relationships between leaf traits, climate and soil nutrient fertility, where N_{mass} was more dependent on soil fertility than on light or other climatic factors (Ordonez et al., 2009).

Although *LMA* and N_{mass} are both physiological determinants of N_{area}, this trait was more strongly associated with *LMA* as demonstrated in other plant species (Wright et al., 2004); this phenomenon reflects a trade-off between the interaction between N supply and light interception with the potential for photosynthesis per unit of area (A_{n}). The A_{n} per unit of leaf area depends on N_{area}, with variations in the photosynthetic capacity for a given N_{area} among species (Evans, 1989). In our work, A_{n} was strongly correlated with N_{area} in an asymptotical relationship (Fig. 2.3b), as observed in a wide diversity of other crops including sunflower (*Helianthus annuus*), kenaf (*Hibiscus cannabinus*), and cynara (*Cynara cardunculus*) (Archontoulis et al., 2012; Trapani and Hall, 1996), but in contrast with a linear trend in *T. aestivum* grown in a hydroponic system (Dreccer et al., 2000c).

In a scenario in which N supply is limiting, N availability is not able to satisfy plant N demand. Thus, reductions in plant height, N uptake, the N partitioned toward the leaves, N_{mass} and leaf area are expected. On the other side, the plant aims to maximize C assimilation maximizing A_{n} which is highly correlated with N_{area}. *LMA* was more sensitive to the interaction between N supply and plant population density than to these factors independently.

2.4.3. Implications of the present results and further research

It is well known that the amount of N fertilizer and plant population density regulate canopy architecture and photosynthesis, affecting C accumulation and allocation between stems and reproductive organs, further influencing stem mechanical strength and final seed yield (Dordas, 2009; Kuai et al., 2016). Although high density leads to limited growth and smaller photosynthetic area per plant, the change of LAI, IPAR and RUE, before flowering is the foundation of crop yield (Kuai et al., 2016). However, studies evaluating the interaction between both agronomical practices, i.e. N supply and planting density, on N distribution and leaf traits in oilseed rape or any other crop are scarce.

The results of this study suggest that the interaction between N and light determine N distribution and leaf architectural traits, which could lead to modifications in the light environment with potential implications for C assimilation at plant level. In addition to the effects on N distribution and leaf traits, N supply and plant population density have been related to stem branching patterns in *Arabidopsis thaliana*, *Solanum tuberosum* and *Z. mays*, which shape overall plant architecture (de Jong et al., 2014; Postma et al., 2014; Vos, 1995). We hypothesize that N availability interacting with light interception, determines N distribution and defines stem branching patterns at phytomer and plant level. To test this hypothesis further studies are required, taking into account that N and C economies are dynamic processes (Gastal et al., 2015; Gombert et al., 2010; Malagoli et al., 2005), whereas the interaction among environmental factors and plant architecture should be addressed and scaled up from the leaf level to organization levels relevant for ecophysiology and agronomy (i.e., population level).

In conclusion, we have shown that the interaction between N supply and plant population density played a major role in altering N per unit of area (N_{area}), which is the main determinant of photosynthesis (A_{n}). This interaction also affects LMA, while N supply determines N content per unit leaf mass (N_{mass}). These results suggest that the interaction between N supply and plant population density has significant effects on both N distribution and leaf mass per area, which together with effects on plant height, could have important implications for light distribution and therefore for C assimilation at plant level. Further studies taking into account effects of the interaction between N supply and plant population density on organ and plant architecture over time, are needed to scale up from leaf level to levels relevant for crop physiology and breeding programmes.

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Chapter 3

Effect of nitrogen availability on assimilate source/sink ratio, branching, and silique production in oilseed rape. An analysis combining experimental and functional-structural modelling approaches

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Abstract

Understanding the response of plant physiological and architectural traits to cultural practices such as nutrient supply and plant density is crucial for designing a crop ideotype. Functional-structural plant (FSP) modelling offers excellent opportunities to quantify most of these traits, while considering interactions among organs. By combining experimental and modelling approaches, this study evaluated i) the importance of N supply as determinant of the source/sink ratio at plant level, and its potential effect on bud outgrowth, and ii) the effect of the number of branches per plant on the source/sink ratio during the reproductive stage, and the subsequent impact on silique production

The experiment combined two contrasting rates of N supplies and two plant population densities. As result, different number of branches and siliques per plant were obtained. N supply had a major impact on C assimilation, with maximum leaf and silique photosynthesis of 16% and 87% respectively at higher at high N supply than at low N. Since the number of branches and siliques per plant simulated by FSP modelling approach were similar to the measured numbers, our hypothesized mechanism that the number of siliques is determined by the source/sink ratio, which in itself is affected by the number of branches, was confirmed. N availability determinates bud outgrowth through modifying C assimilation and thus the source/sink ratio. The modelling approach also showed the main raceme of an oilseed rape plant has the potential to develop a similar number of siliques as a plant with several branches. However, this cannot be reached due to multiple factors here discussed.

Additional keywords: branching, *Brassica napus*, bud outgrowth, FSP modelling, nitrogen, siliques, source/sink

3.1. Introduction

Donald (1968) defined "crop ideotype" as "a plant model expected to yield a greater quantity of grain, oil or other useful products when developed as a cultivar in a specific agronomic environment". The search for such an ideotype has focused the attention of crop physiologists and breeders on morphological traits as emergents of physiological processes to create cultivars with high agronomic performance (Foulkes et al., 2011; Thurling, 1991). In oilseed rape (*Brassica napus*), stem branching pattern is a key trait of such a crop ideotype: the number, size and spatial distribution of branches determining source and sink dynamics (Jullien et al., 2011; Pinet et al., 2015), and therefore grain yield. Plant stems are composed of phytomers assembled along a vertical axis. Each phytomer consists of a leaf, a stem segment, and an axillary bud (McSteen and Leyser, 2005). Branches grow from axillary buds located on the stem. Stem branching depends on the number and positioning of axillary buds along the stem (Wang and Li, 2008). The factors that trigger bud outgrowth can be classified into endogenous and exogenous factors, which are in active interaction. Intensity of photosynthetically active radiation (PAR) driving plant assimilate availability, has been identified as an important determinant of bud outgrowth (Leduc et al., 2014). This factor explains most of the variation in branch number in crops such as sorghum (*Sorghum bicolor*) (Kim et al., 2010) or rice (*Oryza sativa*) (Luquet et al., 2006). Another factor is the red: far red ratio, a signal for presence of surrounding vegetation that triggers shade avoidance responses in plants (Ballaré et al., 1990). In crops such as wheat (*Triticum aestivum*) (Evers et al., 2006) and oilseed rape (Rondanini et al., 2014), a low red:far-red ratio has been linked to suppression of bud outgrowth. It is also well known that branching is modulated by hormones such as auxins and strigolactones (Domagalska and Leyser, 2011), and cytokinins (Rameau et al., 2015), which, in interaction with sugar signalling, determine bud outgrowth. However, Mason et al. (2014) proposed that auxin depletion and hormone balance are not sufficient to trigger bud outgrowth, while the source of assimilates appear to be even more important, supporting that branch outgrowth is mostly determined by the sink-source ratio.

Studies evaluating the effect of carbon (C) availability or sugar signalling on bud outgrowth, should also consider that these signals interact with nitrogen (N) signalling pathways (Osuna et al., 2015). Associations between branching and N fertilizer input have been found in several crops such as potato (*Solanum tuberosum*) (Vos, 1995), wheat (Longnecker et al., 1993), maize (*Zea mays*) (Sangoi et al., 2011), and oilseed rape (Jankowski et al., 2016), suggesting that N availability plays a key role in branch outgrowth. N is involved in light interception and C assimilation (Hikosaka et al., 2016) and meristematic activities (de Jong et al., 2014), but there is no conclusive evidence of a direct role for nutrients in bud outgrowth (Rameau et al., 2015). In oilseed rape, branches are the structures where the siliques develop, and, therefore, the foundation is laid for grain yield (Kirkegaard et al., 2018). However, the role of resource allocation to developing siliques remains poorly understood (Bennett et al., 2011). The development of reproductive buds begin during the vegetative stage (Meier, 2001), and their transition from reproductive bud-to-flower will only happen if plant carbon (C) reserves are high enough to trigger this transition (Poethig, 2010; Wingler, 2018). If carbon reserves are insufficient, flowers will abort or wither (Wingler, 2018), and obviously they will never develop into silique. During the reproductive stage, the number of branches per plant also determines the assimilate source/sink ratio (Pinet et al., 2015; Wang et al., 2011), affecting the assimilate partitioning among reproductive organs, and the number of siliques per plant (Drechsler et al., 2016; Pinet et al., 2015). To what extent N supply could determine branching through an effect on C assimilation, and the consequences this may have for silique production, remain to be quantified.

Because silique development follows a basipetal pattern along the main stem, while silique setting follows an acropetal pattern (Gombert et al., 2010), exploring interactions between branching and silique development purely based on experimentation cannot be done experimentally alone. Functional-structural plant (FSP) modelling can be used to evaluate the impact of N supply on plant architecture and on yield formation as it considers feedbacks at organ and plant level and can predict yield formation based on the underlying processes. FSP modelling "explicitly describes the development over time of the three-dimensional (3D) architecture of plants, as governed by physiological processes that, in turn, depends on environmental factors" (Evers and Marcelis, 2019; Vos et al., 2010). Most of the current FSP models account for C assimilation provided by photosynthetic organs, while assimilate partitioning is regulated based on the source-sink approach (Evers and Bastiaans, 2016; Wubs et al., 2013). Therefore, an FSP modelling approach provides excellent opportunities to quantify and analyse the relationship between N supply and C dynamics, in relation to plant architecture and yield formation in oilseed rape. This study aims at evaluating i) the importance of N supply as determinant of the source/sink ratio at plant level, and its potential effect on branch outgrowth, and ii) the effect of the number of branches per plant on the source/sink ratio during the reproductive stage, and the subsequent impact on silique production. In this way, by combining experimental and modelling approaches, this study wants to improve the understanding of some poorly understood or insufficiently quantified physiological aspects associated to stem branching and silique development.

3.2. Materials and methods

To address our aims, an experiment was performed to gather data on oilseed rape growth and development at different levels of N availability. This information was used for model calibration, to evaluate the relationship between source/sink ratio and stem branching in response to N supply, and the final impact on silique formation in oilseed rape.

3.2.1. Experimental setup

Spring oilseed rape cv. ‘Solar CL’ (NPZ-Lembke, Germany) was sown in containers (0.7 × 0.9 × 0.4 m) under an open-sided shelter, in Wageningen, the Netherlands (51° 58′ N, 5° 40′ E). The experiment was sown on April 17 and harvested on August 7 of 2013 and the treatments consisted of combinations of two rates of N supplies: 50 (N50) and 150 (N150) kg ha^{-1} and two plant population densities: 50 (D50) and 150 (D150) plants m^{-2}. Details on the experimental set-up can be found in Chapter 2. Briefly, the containers were arranged closely together to ensure canopy homogeneity, and twelve guard containers were placed around the experimental containers aimed to avoid border effects on the plants that were to be measured. As in previous experiments (Evers et al., 2006; Lyons et al., 2009; Retuerto et al., 1996), treatments were assessed in a container and, in agreement with the homogeneity of soil fertility the soil (coefficient of variation of N present in the soil at sowing was 3.74%) and of the plant population in this well-managed experiment. This allowed very high similarity across each experimental unit. Containers were re-arranged two times to reduce position effects in the open-sided shelter. Phenological growth stages in oilseed rape were recorded according to the BBCH phenological scale (Meier, 2001).

3.2.2. Weather data and soil nutrients

Air temperature was recorded (Datataker DT600, Data taker Data Loggers, Cambridgeshire, UK) with shielded thermocouples (type T, TempControl Industrial Electronic Products, Voorburg, the Netherlands) placed at mid-canopy, at 15 min intervals from sowing to physiological maturity. To the sandy soil containing 30 kg N ha^{-1} and 3.1% organic material,

$Ca(H_2PO_4)_2$ and $K_2SO_4.MgSO_4$ were applied at sowing. Fertilizer KAS-27 (13.5% NO_3 and 13.5% NH_4) was applied to reach 50 and 150 kg N ha^{-1}. In the lower N treatment 50 kg N ha^{-1} was applied 2 days after emergence, while in the higher N treatment 150 kg N ha^{-1} was split into two applications: 100 kg ha^{-1} 2 days after emergence (BBCH 10) and 50 kg ha^{-1} when the 5th internode was expanded (BBCH 35).

3.2.3. Plant measurements

Five days after emergence, 30 representative plants per treatment (6 plants per container located in the middle of each treatment), were randomly selected and tagged with plastic rings. Destructive sampling was performed at the following phenological stages: (1) rosette (BBCH 30), (2) flower buds visible but still closed or budding (BBCH 50), (3) first flowers open (BBCH 60), (4) 50% flowers on main raceme were open, older petals falling (BBCH 65) (5) 50% of siliques reached final size (BBCH 75), and (6) physiological maturity (BBCH 89). At each sampling, 5 plants per treatment (previously tagged), were harvested and leaf area (LI 3100; LI-COR, Lincoln, Nebraska, USA) and geometry of individual leaves were recorded. The number of vegetative and reproductive phytomers was recorded, whereas their length, diameter and branch angle were measured using a ruler, digital callipers and protractor, respectively. Dry matter of leaves, stems, branches, flowers and siliques was recorded after oven drying at 70 ºC for 48 hours. N content of leaves and stems were measured by the combustion method of Dumas (Flash 2000, Thermo Fisher Scientific Inc., Delft, the Netherlands). With this information, N_{area} (N content per area), *LMA* (leaf mass per area), and stem N content were calculated. Leaf growth duration, flower growth duration and silique maturity were recorded twice a week by non-destructive measurements. Photosynthesis rate of green leaves and siliques was measured 7 days before and 27 days after flowering respectively, with a Li-COR 6400XT Portable Photosynthesis System (LI-COR, Lincoln, Nebraska, USA), at saturated incident light on surface area (I_{inc}) (I_{inc}=1500 µmol m^{-2} s^{-1}; CO_2=390 µmol mol^{-1}) and at a temperature of 25 °C.

3.2.4. Model development and adaptation

To assess the effect of N on oilseed rape architecture and its impact on branch and productivity, a generic FSP model, developed for black mustard (*Brassica nigra*) (de Vries et al., 2018) and implemented in the platform GroImp v1.5. GroIMP (Hemmerling et al., 2008), was adapted to oilseed rape. The FSP model for oilseed rape is based on Relational Growth Grammars (RGG), a graph-based extension of the L-system formalism, which uses the XL modelling language

(Hemmerling et al., 2008). In this model, the oilseed rape plant (Fig. 3.1a) is a modular structure represented as a compilation of phytomers (Fig. 3.1b). Internodes, petioles, flowers and siliques were represented as cylinders, while leaves geometry was digitalized using PlotDigitizer software (https://sourceforge.net/projects/plotdigitizer/), and the point cloud representing the leaf shape, was converted into a 3D mesh surface. This approach captures the shape and curve of oilseed rape leaves in a realistic way. Phyllochron, plastochron and the life span of cotyledons, leaves, flowers and siliques were input parameters derived from our experimental data and literature (Gabrielle et al., 1998) (Table 3.1). Plant functionality was modelled based on C assimilation, flow of assimilates, sink strength and the trade-off among organs, simulated at organ level (Evers, 2016).

3.2.5. *Environmental input*

The light environment experienced by each plant organ was simulated using a virtual dome containing 24-point sources of both diffuse and direct light sources spread over the solar path that takes latitude and the simulated day of year into account (Evers et al., 2010). Distribution of light in 3D over the different plant parts was calculated using the ray tracer principles outlined in Hemmerling et al. (2008), while time was expressed in thermal time units, calculated by summing daily mean temperatures, using a base temperature of 0°C (Gabrielle et al., 1998).

3.2.6. *Organ sink strength*

Assimilates demand of leaves, flowers and siliques was regulated by their sink strength, i.e. their potential growth rate. The potential growth rate (c_{m}) of each organ was calculated by the beta growth equation (Yin et al., 2003):

$$c_{\mathrm{m}} = \frac{2t_{\mathrm{e}} - t_{\mathrm{m}}}{t_{\mathrm{e}}(t_{\mathrm{e}} - t_{\mathrm{m}})}\left(\frac{t_{\mathrm{m}}}{t_{\mathrm{e}}}\right)^{\frac{t_{\mathrm{m}}}{t_{\mathrm{e}}-t_{\mathrm{m}}}} w_{\mathrm{max}} \qquad \text{(Eq. 3.1)}$$

where w_{max} is the maximum length or diameter of the organ (m), t_{e} is the time when the organ stops its growth (°C day), while t_{m} is the moment when the maximum growth rate is reached (°C day).

3.2.7. *Carbon assimilation*

C assimilation was calculated from the amount of intercepted PAR (Hikosaka et al., 2016;

Niinemets and Anten, 2009) by green leaves and siliques (Muller and Diepenbrock, 2006). In this way, the gross photosynthesis (A_{gross}) of each leaf and silique was calculated as:

$$A_{\text{gross}} = A_{\text{max}}\left(1 - e^{\frac{-eff \times PAR}{A_{\text{max}}}}\right) \qquad (\text{Eq. } 3.2)$$

where A_{max} is the maximum rate of photosynthesis at saturating irradiance (μmol CO_2 m^{-2} s^{-1}), *eff* is the light use efficiency of C3 plants, and PAR corresponds to the photosynthetically active radiation (μmol m^{-2} s^{-1}) intercepted by the leaf or the silique. The photosynthetic capacity of leaves and siliques (A_{max}) was calculated as:

$$A_{\text{max}} = A_{\text{max0}} \times \%PAR^{0.4} \qquad (\text{Eq. } 3.3)$$

where A_{max0} is the maximum rate of photosynthesis at saturating irradiance (μmol CO_2 m^{-2} s^{-1}), $\%PAR$ is the proportion of photosynthetically active radiation (PAR) intercepted by the leaf or silique (%), while 0.4 is an empirical coefficient relating the PAR gradient in the canopy to the gradient in A_{max} (Niinemets and Anten, 2009). The model also assumes that N supply turns into a limiting factor only after two expanded leaves (BBCH 12), a stage that in our experiment corresponded to 233.4 °C day. Therefore, from plant emergence until BBCH 12, the same $Amax_0$ was used for leaves and siliques growing under different N supply, and only after 233.4 °C day different input parameters for A_{max0} were used for high and low N supply.

3.2.8. Organ growth determined by source and sink approach

The model assumes that leaves and green siliques are the only "source" of photo-assimilates, which determines the plant C status, represented as a common pool (B_{Net}) of assimilates distributed among all competing plant organs. The allocation of these assimilates depends on the relative sink strength, defined by Heuvelink (1996) as the assimilates needed to achieve potential growth (sink) relative to the sum of sink strengths of all growing organs in the plant. If assimilates available to the organ were higher than the organ sink strength, the difference was added to the plant common pool for the next time step (Evers et al., 2010). Assimilate allocation to the stem was modelled by a pipe model (de Vries et al., 2018), where the sink strength of the stem was determined by the potential thickness of the internodes.

Table 3.1. Input parameters, definition, value and origin

Parameter	Meaning	Value	Origin
$Amax_0$	Leaf photosynthesis at light saturation (µmol CO_2 m^{-2} s^{-1})		Experiment
	- High N-Low density	23.95	
	- High N-High density	21.58	
	- Low N-Low density	21.39	
	- Low N-High density	17.96	
baseLW	Length/width ratio	2.2061	Experiment
BaseT	Base temperature (°C)	0	(Gabrielle et al., 1998)
BranchAngle	Branch insertion angle (°)		Experiment
	- High density	25.5	
	- Low density	40.5	
Cot_Wmax	Maximal cotyledon length (m)	0.048	Experiment
F_te	Time from flower initiation to final silique length (°C day)	839	Optimized
fMax	Maximum flower length (m)	0.10	Experiment
itnMean	Mean internode length (m)	0.0639	Experiment
I_te	Growth Duration Parameter for internodes (°C day)	380	Optimized
I_tm	Time for maximum growth rate (°C day)	190	Optimized
LeafApprt	Time for the development of each phytomer (days)	29.9	Experiment
LMA	Leaf mass per area (g m^{-2})	68.8	Experiment
LMax	Maximum leaf length (m)	0.28	Experiment
L_te	Phyllochron (°C day)	287	Experiment
SSmin	Source/sink ratio needed to trigger branching		Optimized
	- High density	0.78	
	- Low density	1.17	
NMass	N content of leaves per unit mass (g N g^{-1} leaf)	0.04788	Experiment
PhytomerMean	Number of vegetative phytomers	14	Experiment
TotalPhytomerMean	Total number of phytomers (vegetative = reproductive)	200	Optimized
SeedWeight	Seed weight (g)	0.004	Experiment
$SiliqueAmax_0$	Silique photosynthesis at light saturation (µmol CO_2 m^{-2} s^{-1})		Experiment
	- High N-Low density	5.93	
	- High N-High density	5.11	
	- Low N-Low density	2.71	
	- Low N-High density	3.19	

3.2.9. Branch and silique outgrowth

In the FSP model for oilseed rape, the main stem is represented as a collection of 14 vegetative phytomers sequentially produced, after which the meristem produces reproductive phytomers. Each vegetative phytomer has an axillary meristem with the potential to develop in a lateral branch by producing a new sequence of vegetative and reproductive phytomers. The critical moment for branch outgrowth takes place when the plant begins the differentiation of flower buds on top of the main raceme, but such flower buds are still enclosed by the leaves (BBCH 50), as observed in the current experiment and described in the literature (de Jong et al., 2014), after which production of vegetative phytomers stops. In the model, at this phenological stage a number of axillary buds will break their dormancy according to the plant C status, represented by the source/sink ratio at plant level (*R*) at the budding stage (BBCH 50):

$$R = B_{\mathrm{Net}} \Big/ \sum_{i=1}^{n} D_{\mathrm{i}} \qquad \text{(Eq. 3.4)}$$

where B_{Net} is the pool of assimilates available for growth at BBCH 50, *n* is the total number of sink organs in the plant at that stage, and D_{i} is the sink strength of each growing organ. The number of axillary buds ($Branch_{\mathrm{n}}$) allowed to grow out is calculated from *R* at BBCH 50:

$$Branch_{\mathrm{n}} = \frac{B_{\mathrm{m}} - 1}{R_{\mathrm{m}} - R_1}\left(R_{\mathrm{B50}} - R_1 + \frac{R_{\mathrm{m}} - R_1}{B_{\mathrm{m}} - 1}\right) \qquad \text{(Eq. 3.5)}$$

where R_1 is the source/sink ratio needed for 1 branch, R_{m} the source/sink ratio needed for maximum branch number and B_{m} is the maximum branch number.

In an oilseed rape plant, each reproductive phytomer holds a reproductive bud, which could turn into a flower and subsequently a silique (Jullien et al., 2011). In our model, we assume the transition from each flower-to-silique is decided based on the source/sink ratio at plant level (*R*) 340 °C day after flower bud formation (Eq. 3.4). The development of these flowers follows an acropetal pattern, where reproductive buds located on the bottom of the branch develop first, followed in sequential order by the buds located at a higher position. We assume that if the plant C status is higher than a threshold value at that flower age, transition to a silique occurs. This decision is made for each flower individually as it appears. So, the flower will continue its development to become a silique, only if the source/sink ratio at plant level is higher than the source/sink ratio needed to develop a silique. Flower initiation and silique initiation start on the main raceme and propagate basipetally to the lateral branches, but they propagate in an acropetal sequence within each branch (Jullien et al., 2011). The model also assumes that plant C status is the only driver of silique development; limitation of growth by water, or other nutrients than N were not considered. Silique fertility is highly dependent on entomophilous pollination by insects like honeybees (Abrol, 2007; Chambo et al., 2014), but this factor was also not considered in our approach. An elaborate technical description of the implementation of carbon assimilation, allocation and organ growth can be found in de Vries et al. (2018).

3.2.10. Simulations

Plant development and growth were simulated from emergence until physiological maturity at a daily time step, in a grid of 3 × 3 oilseed rape plants (Fig. 3.1c) for all treatments (Fig. 3.A1). The plot was cloned 20 × 20 times to minimize any border effects. Biomass accumulation, leaf area index (LAI), intercepted PAR, source/sink ratio over time, number of branches per plant, and number of siliques per branch, plant and per unit of area, were simulated under two N supplies: 50 (N50) and 150 (N150) kg ha^{-1} and two plant population densities: 50 (D50) and 150 (D150) plants m^{-2}.

3.2.11. Model evaluation

The dynamics of plant biomass, LAI and intercepted PAR were simulated for oilseed rape by the FSP model. The differences between measured data and model output were assessed by the normalized Root Mean Square Error (RMSE), defined as the standard deviation of the residuals, considering the range of the measured data. This estimator was chosen because it allows to compare dynamics with different scales (i.e. in this case, treatments with highest and lowest biomass, LAI, etc.). Source/sink ratio at plant level was simulated from plant emergence until physiological maturity.

3.2.12. Evaluating the contribution of branching to silique production

The treatments N150-D50 and N150-D150 were used to determine the value of the threshold source/sink ratio needed to calculate number of branches, and this threshold value was used to simulate the number of branches per plant in the treatments N50-D50 and N50-D150. While the number of siliques per plant was determined by optimizing from the source/sink ratio threshold needed to calculate silique fate in N150-D50, the treatment with the highest availability of nutrients and light. This value was used to simulate the number of siliques per plant in the treatments N150-D150, N50-D50 and N50-D150. The model outputs were number of branches per plant and number of siliques per plant, which were compared with values observed in the experiment.

The impact of branching on the source/sink ratio after budding, and subsequently on the number of siliques per plant, was evaluated for all the four treatments mentioned before by manipulating the parameter maximum number of branches in the model. For this purpose, the source/sink and siliques per plant were simulated by fixing the maximum number of lateral branches to zero (i.e. only the main raceme can develop and produces siliques), mimicking branch pruning, and these results were compared with "normal plants branching", where the maximum number of branches including lateral branches and main raceme was equal to 14.

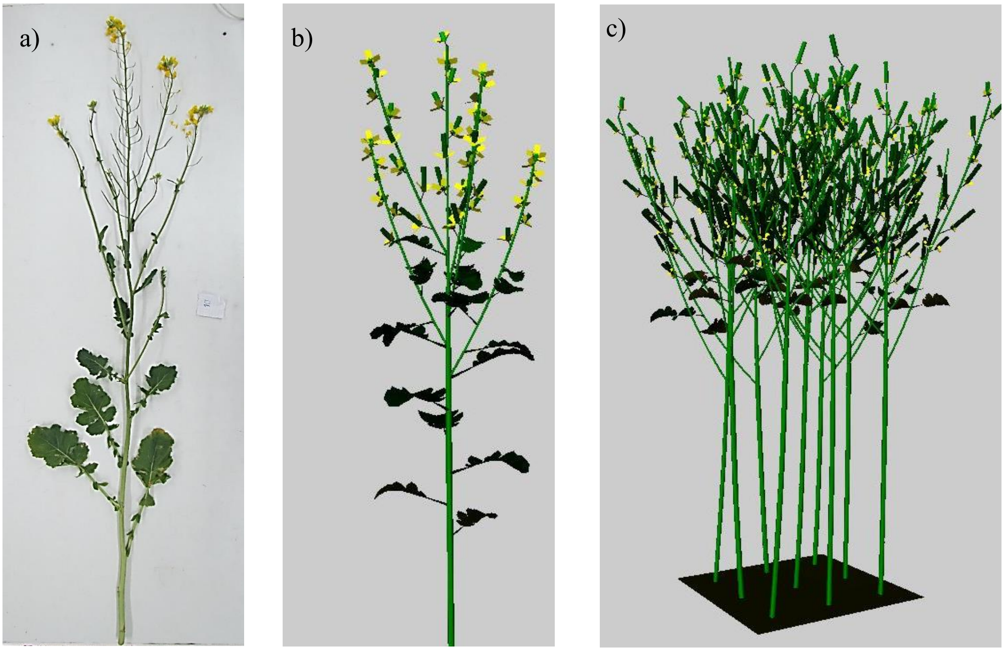

Fig. 3.1. a) Picture of an oilseed rape plant 58 days after plant emergence, b) virtual plant from treatment N150-D50, and c) plant stand in a grid of 3 × 3, simulated by FSP modelling.

3.3. Results

3.3.1. Experimental results

The phenological stages of emergence (BBCH 10), budding (BBCH 50), and beginning of flowering (BBCH 60) and full flowering (BBCH 65) were not affected ($P>0.05$) by the treatments (data not shown). However, the moment of physiological maturity (BBCH 89) was affected ($P<0.001$) by the effect of plant population and the interaction between N supply and plant population (Table 3.2): N50-D150 reached physiological maturity 125, 157, and 260 degree day (DD) earlier than the treatments N50-D50, N150-D50 and N150-D150, respectively. The maximum leaf photosynthesis at light saturation (A_{max0}) at high N supply was 16% higher ($P<0.05$) than at low N. At early grain filling, the A_{max0} of siliques was 87% higher at high N supply than in treatments with low N. The number of branches at physiological maturity was affected by both N supply and plant density ($P<0.001$). Plants in treatment N150-D50 produced on average 5.1 branches, while 2.7 and 2.9 branches were recorded in N150-D150 and N50-D50, respectively. Only 1.4 branches were produced under the most limiting conditions, N50-D150. The number of siliques per plant produced by N150-D50 was twice as high as that of N50-D50, ca. 3 times as high as that of N150-D150 and more than 4 times as high as that of N50-D150 ($P<0.05$) (Table 3.2).

Table 3.2. Maximum leaf and silique photosynthesis ($Amax_0$), number of branches per plant at maturity, number of siliques per plant and thermal time until physiological maturity, as affected by N supply (N) and plant density (D)

Treatments		$Amax_0$ leaf (μmol CO_2 m^{-2} s^{-1})	$Amax_0$ silique (μmol CO_2 m^{-2} s^{-1})	Branches (branches per plant)	Siliques (siliques per plant)	Physiological maturity (DD)
N150	D50	24.0 ± 1.6	5.9 ± 0.7	5.1 ± 0.3	105 ± 5.6	1572 ± 7.1
	D150	21.6 ± 1.3	5.1 ± 1.0	2.7 ± 0.4	36 ± 3.8	1697 ± 4.3
N50	D50	21.4 ± 2.8	2.7 ± 0.4	2.9 ± 0.1	55 ± 3.6	1729 ± 5.3
	D150	18.0 ± 2.6	3.2 ± 0.3	1.4 ± 0.4	24 ± 2.1	1832 ± 9.1
	N	*	*	***	***	NS
	D	NS	NS	***	***	***
	N × D	NS	NS	NS	***	***

Asterisks indicate significant differences between treatments at *$P<0.05$ and ***$P<0.001$, while NS = not significant ($P>0.05$), according to ANOVA.

N150 = 150 kg N ha^{-1}, N50 = 50 kg N ha^{-1}, D50 = 50 plants m^{-2}, D150 = 150 plants m^{-2}

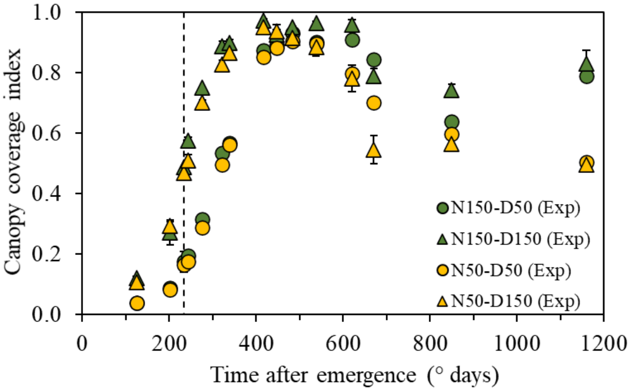

Fig. 3.2. Canopy coverage index observed in the experiment (Exp), in response to N supply and plant density. Dashed line at 233.4 DD represents 2 expanded leaves (BBCH 12). Vertical bars indicate standard error of means.

Differences in canopy coverage index in response to plant population density (Fig. 3.2) were observed from plant emergence, where canopy growing under high population density (D150) showed a faster increase in coverage reaching than those at lower density (D50). The effects of N supply were observed only after 233 DD, when high N showed a higher canopy coverage than low N. Later, the treatments growing at high N maintained the high canopy coverage, while an earlier and faster decline was observed in the treatments at low N supply (Fig. 3.2). This decrease in canopy coverage index coincided with the end of the vegetative stage, and the onset of leaf senescence.

3.3.2. Model evaluation

The first step in the model evaluation was the assessment of biomass per plant (Fig. 3.3a). In the experiment, the highest biomass per plant was observed in the sampling at the beginning of grain filling (891 DD). At this stage, the biomass of the treatments N50-D50, N150-D150 and N50-D150 were 28, 65 and 76% lower ($P<0.05$) than the biomass at N150-D50, which showed the highest biomass per plant, followed by a quick decline to a stable final value. The biomass of treatments at high plant density were lower than the biomass at low plant density ($P<0.05$), and the peak in biomass was reached earlier for the high plant density, at beginning of flowering (671 DD). A similar pattern was observed in the model (Fig. 3.3a), but the peak in biomass was delayed by ca. 80 DD. The model could not simulate the drop in biomass observed in all the treatments at 767 DD (BBCH 65). This steep reduction in biomass, was coincident with a reducton in stem dry weight, and the development of flowers and siliques during the reproductive stage.

The second step was the assessment of leaf area index (LAI) (Fig. 3.3b). At rosette stage (i.e. 353 DD), the LAI of the treatments at high plant density was higher ($P<0.05$) than at low plant density, while the effects of N supply were only evident after budding (603 DD). Indeed, the peak values of LAI were reached at this stage for all treatments, with a maximal LAI of ca. 7, observed in N150-D150. A faster leaf senescence and then a quicker decline in LAI ($P<0.05$) was observed at low N than at high N. The model simulated this behaviour, but at early stages underestimated the LAI of the high plant density treatments (Fig 3.3b). This was particularly apparent at rosette stage. This model bias was consistent with the delay in PAR intercepted by the canopy simulated by the model (Fig. 3.3c). At early stages, there was a slow increase in intercepted PAR simulated, causing a delay in reaching the maximum intercepted PAR. After flowering, LAI decline did not result in PAR decline due to developing branches and siliques taking over light capture from the leaves. The model did simulate a minor decline of intercepted PAR, particularly in the treatments under low N supply. This small decline in PAR interception stopped around 1000 DD, i.e. when the siliques reached their maximum size intercepting most of the PAR during grain filling.

3.3.3. Physiological traits associated to stem branching and silique production

Source of photoassimilates, sinks, and source/sink ratio over the time were also simulated. Until 350 DD the sources were rather limited (Fig. 3.4a), mainly because the plants only developed 4 or 5 small leaves until the rosette stage. However, after stem elongation the source increased in all treatments, reaching a peak value the day before budding stage (500 DD). Here, the source at low plant density was four times as high as that at high plant density ($P<0.05$), while the differences between high and low N were close to 70% ($P<0.05$). At this stage a peak in sink strength was also observed (Fig 3.4b). The source/sink at budding was ca. three times higher in the treatments at low than at high density ($P<0.05$), while the source at high N supply was on average 50% higher than at low N supply ($P<0.05$).

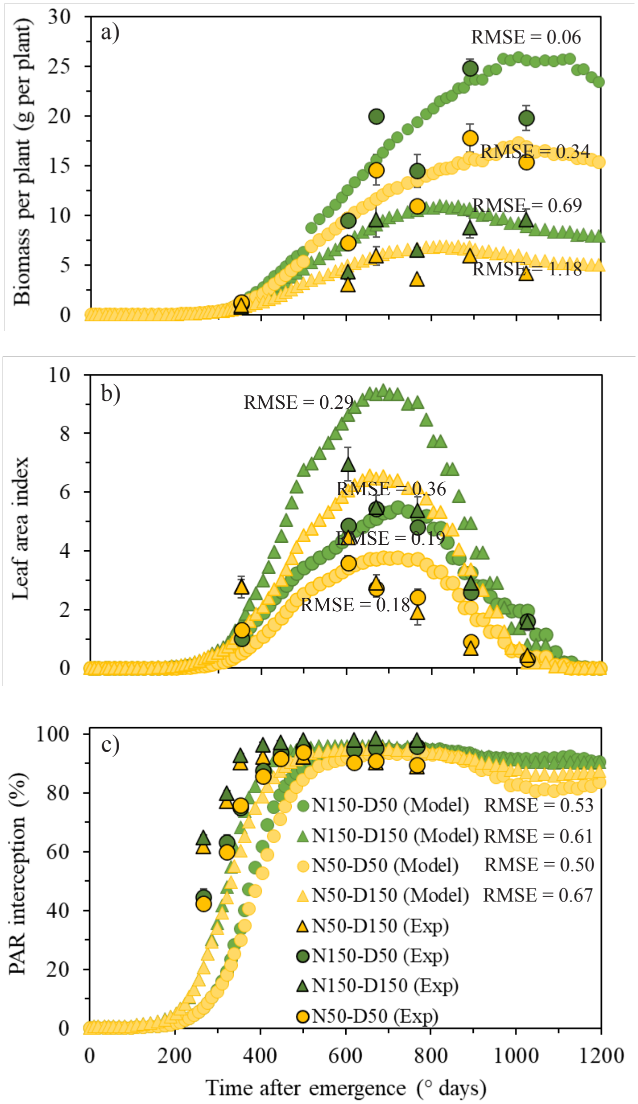

Fig. 3.3. a) Biomass per plant and b) PAR interception of observed data in the experiment (Exp) and simulated values (Model), in response to N supply and plant density. Simulations were performed between the stages plant emergence (BBCH 09) and grain filling (BBCH 79). Vertical bars indicate standard error of means.

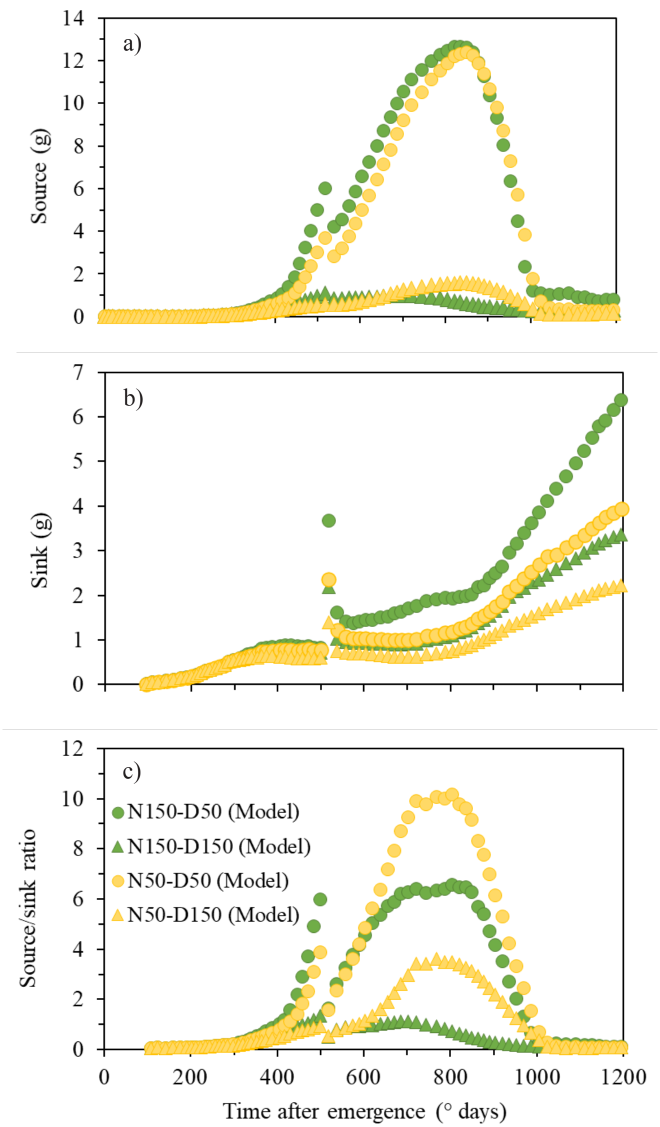

Fig. 3.4. a) Source, b) sink and c) source/sink ratio per plant, in response to N supply and plant density. Simulations were performed between the stages plant emergence (BBCH 09) and grain filling (BBCH 79). Vertical bars indicate standard error of means.

3.3.4. Source/sink ratio as determinant of stem branching

Since the variation in source/sink ratio was mainly explained by plant population density, and to a lesser extent by N supply, the treatments N150-D50 and N150-D150 were selected to calculate the source/sink ratio needed to trigger the branch outgrowth of the first branch. On average, treatments N150-D50 and N150-D150 developed 5.1 and 2.7 branches, respectively (Table 3.2), and according to the simulations the source/sink needed to trigger branching (R_{b50}) was 1.17 and 0.78 respectively (Fig. 3.4). Based on these values of R_{b50}, and Eq. 3.5, a source/sink ratio at budding (BBCH 50) of 3.95 and 5.95 was calculated for the treatments N50-D50 and N50-D150, respectively.

A linear relationship between number of branches and R_{b50} was assumed. This resulted in the observation that a lower number of branches at low N can be explained by the effect of N on photosynthesis and subsequently the source/sink ratio at flowering. However, for different densities different regression lines were needed, indicating that the change in source/sink ratio caused by changing the density cannot fully explain the difference in branching (Fig. 3.5).

3.3.5. Source/sink ratio as determinant of number of siliques

By parameter optimization, we found that 0.12 was the source/sink ratio needed for silique development, and this value was used to simulate the number of siliques in other treatments. Based on our simulations, that treatments N150-D50, N150-D150, N50-D50 and N50-D150 developed 106, 43, 53 and 27 siliques per plant, respectively (Fig. 3.6).

Experimental values were 105, 36, 54 and 24 siliques per plant, respectively. Siliques are photosynthetic organs, and this was implemented in the model. However, the C assimilated by siliques was overestimated by the model producing a late increase in the source/sink ratio with a new increase in number of siliques late in the reproductive stage (data not shown). For indeterminate crops, the beginning and the end of the reproductive stage is not only regulated by endogenous signals (i.e. like source/sink status), but also by their interaction with environmental factors (i.e. photoperiod and light quality), regulating the expression of genes that trigger the beginning and the end of flowering (Ausín et al., 2005; Carmona et al., 2007). These mechanisms should be further incorporated in the model.

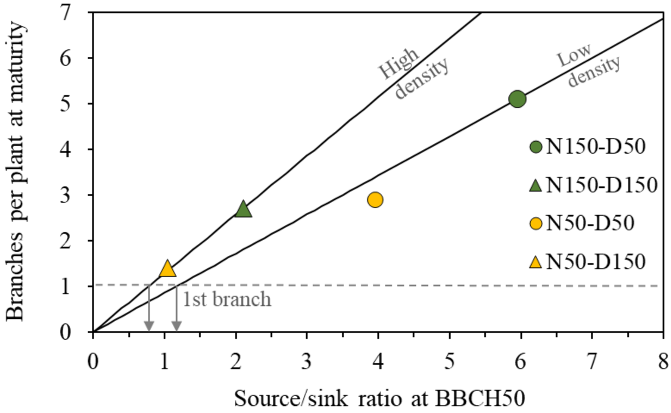

Fig. 3.5. Relationship between number of branches per plant at maturity, and source/sink ratio at budding (BBCH 50).

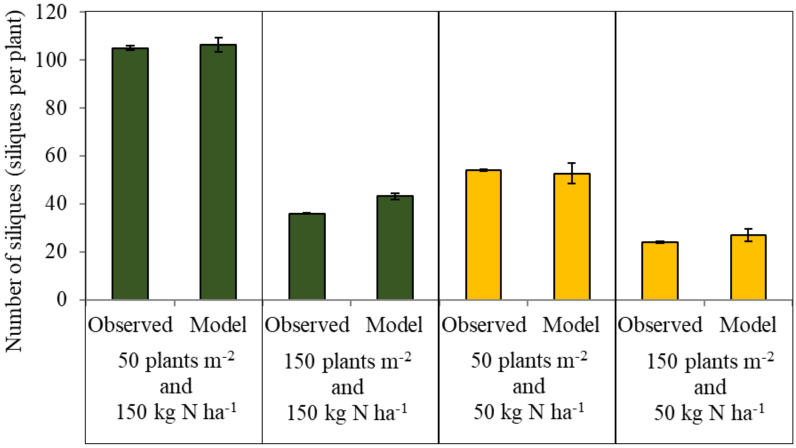

Fig. 3.6. Number of siliques observed and modelled in response to N supply and plant density. Vertical bars indicate standard error of means

3.3.6. Evaluating the contribution of branching to silique production

Setting the maximum number of branches to zero (i.e. the plants can only develop the main raceme) increased the source sink/ratio per plant after budding stage in all treatments (Fig. 3.7), compared to “normal” plants (Fig. 3.4c). This higher availability of resources allowed to compensate for fewer branches per plant by extending the period of grain filling.

For the high plant density treatments and with a maximum number of branches equal to one, the source/sink needed to trigger the development of new siliques remained above the threshold value of 0.18, at 1045 and 1195 DD after emergence. For the low plant density treatments, this ratio remained above the threshold and developing new siliques until ca. 4400 DD. This means that the reproductive phase of plants with only a single branch, grown under low plant density and without any other limitation, could be extended until 240 days, well beyond the 57 days observed in the experiment.

The simulations showed that the treatments N150-D150 and N50-D150 produced 18 and 14 siliques per plant, respectively (Fig. 3.8), while the low population density treatments finally produced 84 siliques per plant.

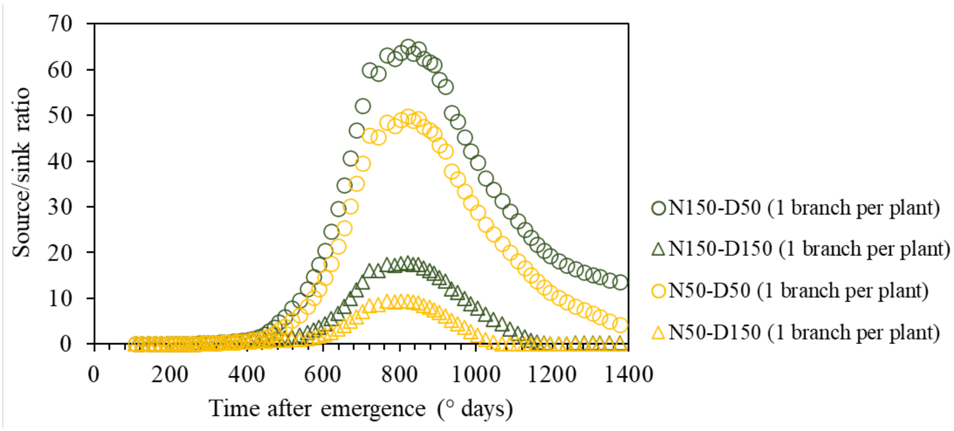

Fig 3.7. Source/sink ratio, for plants with a maximum number of branches equal to 1, in response to N supply and plant density. Simulations were performed between the stages plant emergence (BBCH 09) and grain filling (BBCH 79).

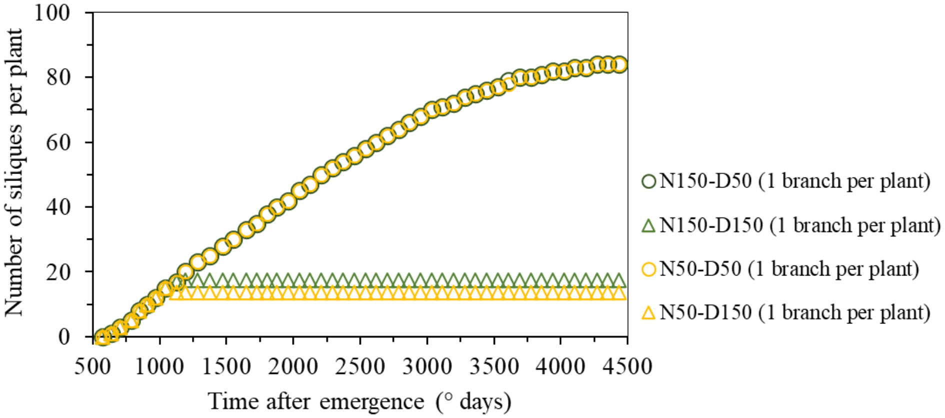

Fig 3.8. Silique number over the time, for plants with a maximum number of branches equal to 1, in response to N supply and plant density.

3.4. Discussion

We hypothesized that N supply determines the number of branches per plant based on its effect on the source/sink ratio, and during the reproductive stage it is the number of branches per plant which determines source/sink, and subsequently the number of siliques per plant. The interaction between branching and silique development was evaluated by combining experimental and modelling approaches, using the model to quantify the source/sink ratio per plant at critical stages of development. Our results and their potential implications are discussed below.

3.4.1. Effect of N availability on C assimilation

Nitrogen supply has a major impact on C assimilation (Evans, 1989). This has been confirmed in the current study where an increase by 16% in maximum leaf photosynthesis and by 87% in maximum silique photosynthesis were observed in response to a threefold increase in N supply. High N availability also delayed leaf senescence, represented by a slower decline in LAI measured after flowering, compared with treatments grown at low N supply. N supply impacts leaf area of oilseed rape mainly at early stages (i.e. budding stage), while the effects of plant population density are more evident at later stages, i.e., between flowering and maturity (Kuai et al., 2016). However, the latter is not supported by our results since leaf nor silique photosynthesis was affected by plant population density.

3.4.2. Effect of N supply and plant density on source/sink ratio and branching

In our FSPM modelling approach, branch outgrowth was implemented as determined by source/sink ratio, mainly driven by light interception. N availability was also determinant of bud outgrowth, but through modifying C assimilation and thus the source/sink ratio. Indeed, the plant source/sink ratio at budding stage was more responsive to variations in plant density than to variations in N supply, being in our study 50% higher at low population density than at high plant density. Therefore, plants at high intraspecific competition trigger branch outgrowth at lower source/sink ratio than at lower intraspecific competition.

The relationship between the number of branches per plant and the source/sink ratio at budding (Fig. 3.5) revealed that next to the effect of N on C assimilation, an additional factor or factors that change with population density are likely to be involved in determining bud outgrowth, not considered in our approach. For instance, both C and N have an additional effect as signalling molecules. Genetic analyses demonstrate that photoassimilates and C metabolism generate sugar signalling pathways for high carbon availability (including hexokinase-1 [HXK1], trehalose-6-P [T6P], and target of rapamycin [TOR]) and for starvation (including Snf1-related protein kinase-1 [SnRK1] and C/S1 bZIP transcription factors) with interactions between them (Wingler, 2018), and with hormones, modulating plant organ growth, development (Rolland et al., 2006) and stem branching. Auxins and strigolactones are two of such hormones (Domagalska and Leyser, 2011), and their export from active axillary meristems can inhibit the activation of axillary buds, and hence stem branching (de Jong et al., 2014). But independently of the effect on C assimilation, N is also linked to hormone signalling (Mueller and Leyser, 2011; Rameau et al., 2015). It is known that the metabolism and signalling of hormones like cytokinin, are closely associated with N availability (Gu et al., 2018). However, the cytokinin-N interactions involved in sensing soil N status and in regulating canopy development are still not clear (Gu et al., 2018). Evaluating the effects of plant C status in interaction with both nutrient and C signalling should be implemented in a modelling approach, to evaluate the effect of nutrients on stem branching pattern.

3.4.3. Importance of stem branching to silique production

The number of siliques per plant is the basis for grain yield and strongly correlated with both plant C status and sink strength between budding and beginning of silique development (Pinet et al., 2015; Wang et al., 2011; Zhang and Flottmann, 2016). During this window of time, branches (de Jong et al., 2014), reproductive buds and flowers, are also defined (Zhang and Flottmann, 2016), and all these sinks are in constant competition for resources. In our study, the number of branches and siliques simulated by the model were similar to the measured numbers, making our hypothesized mechanism that the number of siliques is determined by the source/sink ratio, which in itself is affected by the number of branches, plausible. These results are supported by experiments performed by Wang et al. (2011), where branches were clipped immediately after their appearance, developing more siliques and seeds per silique than control plants that were not clipped. The authors hypothesized that branch clipping reduced the demand for assimilates in the entire plant, reducing the source/sink at plant level. Therefore, without limitations of light, water or nutrients, oilseed rape could almost compensate for a hypothetical reduction in the number of branches per plant (i.e. through branch clipping), by increasing the source/sink ratio after budding and extending the period of silique formation.

The modelling approach also showed the main raceme of an oilseed rape plant has the potential to develop a similar number of siliques as a plant with several branches. This compensation could only be achieved if the reproductive stage was extended by a factor four (without limitations of water or nutrients). However, in Western Europe the typical growing season for spring rape oilseed starts in spring and ends in July/August, and according to our simulations, the reproductive stage of oilseed rape with inhibited lateral branching should be extended until the end of December (i.e. winter season) in order to achieve a full compensation in siliques per plant. Obviously, this does not occur in farming systems, because flowering induction does not only responds to sugar signalling, but it is also sensitive to long days (Matsoukas et al., 2013), and it is also inhibited under low temperatures during the reproductive stage (Lardon and Triboi-Blondel, 1995). An additional constraint would be that an only and longer main raceme could also lead to problems with lodging (Bennett et al., 2012). Although our approach considered silique production as the only compensatory mechanism in response to variations in C availability, it is known that grain number and grain weight are also plastic traits, and they can vary in response to source/sink ratio manipulations around flowering (Kirkegaard et al., 2018; Labra et al., 2017). These compensatory mechanisms should be considered in further studies evaluating of the importance of branching on yield.

Improving the productivity per plant by maximizing the number of branches per plant can also have negative consequences. For example, a high number of branches per plant induces asynchrony of silique maturity, increasing the losses of grains during harvesting by shattering (Hu et al., 2017). For this purpose and during the last decade, farmers in countries like China are increasing the sowing density up to 60 plants per square meter, reaching maximum LAI and light interception earlier in the season, with thinner plants and fewer branches. This practice synchronizes silique maturity, but with a marked reduction in the number of siliques per plant (Hu et al., 2017). This confirm that more sophisticated tools are needed to design an oilseed plant with maximum C assimilation and optimum plant architecture, to maximize grain yield.

3.4.4. FSP model as a tool to explore interactions between plant architecture and silique production

Since analysing feedback mechanisms among stem branching, source/sink ratio and siliques production in response to N supply and plant population density, merely based on experiments is a complex task, we addressed this objective by combining experiment and modelling approaches. FSP modelling contributed generating quantitative information on physiological processes that are difficult to measure in experiments (i.e. source/sink ratio), and to integrating this information at different levels of biological organization (i.e. phytomer, branch, plant, plant population). Model testing indicated that our FSP model can handle the behaviour of oilseed rape, under contrasting N supply and plant population density. Plant biomass and the growth of photosynthetic organs over the time, represented by increases in LAI, were well simulated by the model for the treatments grown at low plant density. For plants grown at high density, LAI was initially underestimated at rosette stage but overestimated during flowering. Our approach assumed that leaf growth is entirely driven by C assimilation determined by maximum photosynthesis, a parameter that is affected by N supply and plant density. This parameter was measured for leaves at stem elongation and for siliques at the beginning of grain filling. However, it has been demonstrated that environmental factors (i.e. irradiance, temperature) and the demand for photoassimilates are involved in short-term alterations of steady-state photosynthetic activity (Heuvelink et al., 2014; Kaiser et al., 2018). Therefore, model bias in predicting biomass, LAI or source/sink ratio over time could be explained by using a dynamic process like photosynthesis as a static parameter.

It is well known that developmental transitions result in changes in the source-sink relationship, and in the development of new organs (i.e. branches, flowers) creating additional carbon sinks (Wingler, 2018). In the model, this transition was evident at budding stage, when the branches were initiated immediately after the flower buds appearing, when peaks in assimilate source, sink and source/sink ratio were clearly observed (Fig. 3.4a, 3.4b and 3.4c respectively). But because flower buds are initiated before at earlier stage (i.e. during stem elongation) becoming in sink before budding stage, the peaks in source and sinks are expected to be less sharp and more gradual. Because floral bud initiation cannot be observed without dissecting the plant stem, our study considered bud appearing as the moment when was used instead of bud initiation. It is also necessary to consider that according our FSP modelling approach, the number of branches can determine the sink strength. This is highly relevant for the model outcome, because internode thickness was modelled using a pipe model, which determines the sink strength of the internodes through calculating the potential thickness, and this drives assimilate allocation to the stem, which is a major sink that competed for assimilates with leaves and seeds (de Vries et al., 2018).

In summary, FSP modelling approach is a useful tool to explore plant-to-plant dynamic interactions in response to light intensity (Zhu et al., 2015), environmental signals (Bongers et al., 2014), and biotic factors (de Vries et al., 2017), particularly for plants with high phenotypic plasticity such as oilseed rape. Many research questions that deal with crop development, competition, plant/crop physiology, and crop management (i.e. row spacing and density, pruning, spraying of biocides, etc.) require plant architecture to be considered, and therefore can be explored by the FSP modelling (Zhu et al., 2015). Perhaps, parameter requirements is one of the main issues that FSP model users have to overcome (Evers, 2016), because the number of parameters and the type of assessment needed to obtain these values are highly demanding in time in labour, in comparison with other simulation models. Nevertheless, if these obstacles can be overcome, FSP modelling offers a wealth of possibilities in plant science (Evers, 2016).

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Supplementary Material

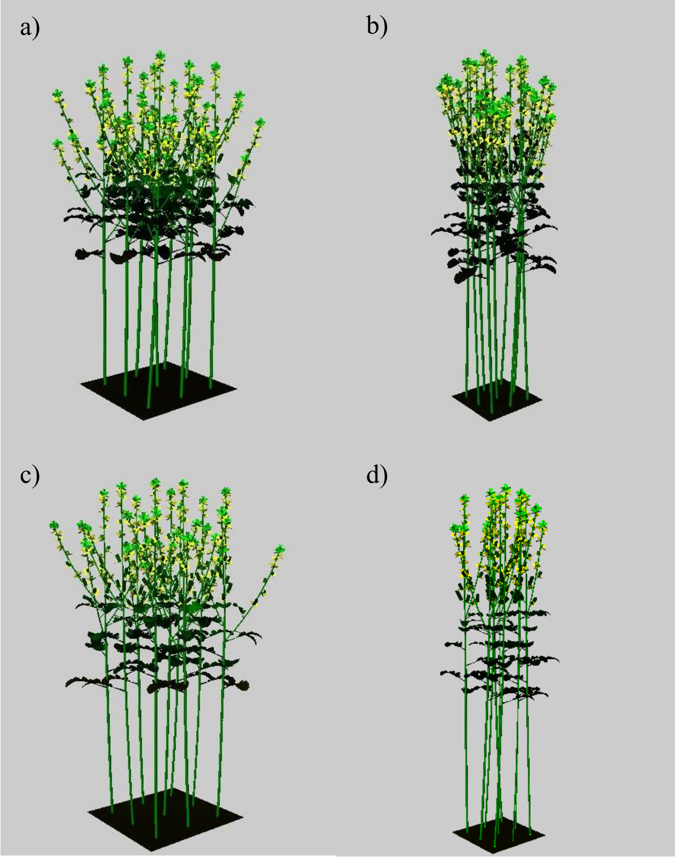

Fig. A3.1. Oilseed rape plants at BBCH 67, in a grid of 3 × 3, in treatments a) N150-D50, b) N150-D150, c) N50-D50 and d) N50-D150, simulated by FSPM modelling.

Chapter 4

Plasticity of seed weight compensates reductions in seed number of oilseed rape in response to shading at flowering

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Published as: Labra M.H., Struik P.C., Evers J.B., Calderini D.F. (2017) Plasticity of seed weight compensates reductions in seed number of oilseed rape in response to shading at flowering. Eur. J. Agron. 84:113-124. DOI: http://dx.doi.org/10.1016/j.eja.2016.12.011

Abstract

Understanding the response of the number of seeds and single-seed weight to the availability of assimilates is crucial for designing breeding strategies aimed to increase seed and oil yield in oilseed rape. This study aims to answer the questions: i) do seed number and seed weight in oilseed rape differ in their plasticity in response to the availability of assimilates at flowering? and ii) how sensitive are oil and protein concentrations to the availability of assimilates during flowering?

A spring oilseed rape hybrid was sown in two field experiments and the treatments were combinations of i) two plant densities and ii) shading or no shading between the beginning and end of flowering. Seed yield was not affected by plant density or by shading. Lower plant density was compensated by an increase in seed number per plant, without effects on single-seed weight. However, the negative effect of shading during flowering on seed number per area was fully compensated by an increase in single-seed weight by 47-61%. The plasticity of single-seed weight observed in the present study of oilseed rape has never been reported for annual seed crops. Shading at flowering increased both the seed filling rate and the duration of the seed filling period at all positions in the canopy. We also observed that the reduction of the source-sink ratio at flowering increasing seed weight does not necessarily modify oil or protein concentrations. Thus, single-seed weight could be targeted to increase seed yield in oilseed rape without compromising oil content.

Additional keywords: *Brassica napus*, phenotypic plasticity, shading, oil, yield component analysis

4.1. Introduction

Food security will be challenged by the growth of the world's population, reaching 9.7 billion people in the next 50 years (United Nations, 2019). The "Green Revolution" improved the yield of energy-dense crops like cereals and oil species, allowing the increase in food calories, fats and proteins consumed by mankind (Khoury et al., 2014). Over the last 50 years, oilseed rape (*Brassica napus* L.), the source of one of the healthiest edible oils for human consumption (Momoh et al., 2004), has increased globally seed production, reaching 71 million tonnes in 2014 (FAOSTAT, 2015). This increase was partially powered by the rising demand for biofuels (Rondanini et al., 2012). Between the years 1991 and 2010 the oilseed yield progress in countries such as China, Canada, India, France and Germany, grew at annual rates in a range between 0.6 and 2% (see Kirkegaard et al. 2016). However, a stagnation has also been seen in countries such as United Kingdom, Brazil, Finland, Sweden and the Czech Republic in recent years (Beddington, 2010; Peltonen-Sainio et al., 2007a; Rondanini et al., 2012). Therefore, new strategies are urgently needed to overcome such increasing leveling-off and to satisfy the growing demand for vegetable oil.

Seed yield is the product of the number of seeds and their average weight (Mitchell, 1970). Since the former is the yield component most associated with seed yield (Peltonen-Sainio et al., 2007b; Sadras and Slafer, 2012), past increase in seed yield has been mainly achieved by increasing the seed number per area (Fischer and Edmeades, 2010). However, modern genotypes of crops like sunflower (*Helianthus annuus*) are reaching seed numbers close to their saturation point from which further increase will not necessarily improve seed yield. Instead, it has been proposed that larger seeds with higher oil concentration, while maintaining seed number, could be a more effective strategy for improving seed and oil yield in oil crops (Pereira et al., 1999).

The time window during which seed number and single-seed weight are established are known to overlap around flowering, but to different degrees depending on the particular crop species (Calderini et al., 2001; Yang et al., 2009). During this period, plants allocate their assimilates to the reproductive organs setting the potential sink capacity (Andrade et al., 2005). Based on the assumption that "each organism has a critical stage in which their energy should be invested into maximizing their potential offspring", Smith and Fretwell (1974) designed a theoretical model that distribute the available energy between size and number of offspring. Using this approach, Sadras (2007) analysed the balance between the seed yield components in grain crops, and hypothesized that seed number is directly related to assimilate availability during flowering, whereas seed weight or size can be considered constant. The plasticity in seed number and the stability of seed weight in response to the availability of assimilates at flowering have been demonstrated for several crop species, including wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), narrow-leafed lupin (*Lupinus angustifolius* L.) and pea (*Pisum sativum* L.) by manipulating the source-sink ratio (Arisnabarreta and Miralles, 2008; Fischer, 1985; Sandaña and Calderini, 2012), supporting the corollary of Sadras (2007). As a consequence, the plasticity of seed number and the conserved response of seed weight to the availability of resources have been assumed for oilseed rape (Diepenbrock, 2000; Gomez and Miralles, 2011). Nevertheless, quantitative evaluations of the sensitivity of yield components at flowering and the potential trade-off between seed number and single-seed weight have been scarcely assessed in oilseed rape (Tayo and Morgan, 1979), and have never been evaluated in field conditions.

A central objective of oilseed rape breeders is to improve oil yield (Labana et al., 2013). The biosynthesis of fatty acids and other components like proteins takes place during the seed filling period (Diepenbrock and Geisler, 1979). Therefore, variations in the rate and length of this phase may affect both seed weight and seed oil concentration. In maize (*Zea mays* L.), seed weight decreased and, on the contrary, the proportion of oil and protein concentration increased in response to lower source-sink ratio during flowering (Jia et al., 2011). Because seed filling and fatty acid synthesis occur simultaneously, a potential trade-off between seed weight and oil concentration deserves special attention (Pereira et al., 1999). Several studies have addressed the effect of the availability of assimilates on oil concentration during seed filling in oilseed rape (Fortescue and Turner, 2007; Iglesias and Miralles, 2014; Rondanini et al., 2014); however, these effects have not been assessed when changes in the source-sink ratio occur at flowering.

Since it has been well established that there is a dynamic interaction between source and sink (Asseng et al., 2016; Calderini et al., 2001; Shi et al., 2016), unravelling the response of yield components to the availability of assimilates during flowering will provide important cues to increase seed and oil production. Therefore, this study aims to answer the following questions: i) do seed number and single-seed weight differ in their plasticity in response to the availability of assimilates at flowering in oilseed rape? and ii) how sensitive are oil and protein concentrations to the availability of assimilates during flowering? To address these questions, we quantified the variations in seed number, seed weight, oil and protein concentrations in response to reduction in incoming photosynthetically active radiation (PAR_{inc}) during flowering (Fig. 4.1) under two contrasting plant densities and in two different growing seasons.

4.2. Materials and methods

4.2.1. Experimental set-up and treatments

Spring oilseed rape cv. ‘Solar CL’ (NPZ-Lembke, Germany) was sown in containers (0.7 × 0.9 × 0.4 m) under an open-sided shelter, in Wageningen, the Netherlands (51° 58′ N, 5° 40′ E). The experiment was sown on April 17 and harvested on August 7 of 2013 and the treatments consisted of combinations of two rates of N supplies: 50 (N50) and 150 (N150) kg ha^{-1} and two plant population densities: 50 (D50) and 150 (D150) plants m^{-2}. Details on the experimental set-up can be found in Chapter 2. Briefly, the containers were arranged closely together to ensure canopy homogeneity, and twelve guard containers were placed around the experimental Spring oilseed rape cv. ‘Solar CL’ (NPZ-Lembke, Germany) was sown in two field experiments carried out at the Estación Experimental Agropecuaria Austral in Valdivia (39º47’ S., 73º14’ W.), Chile, in a Duric Hapludand soil.

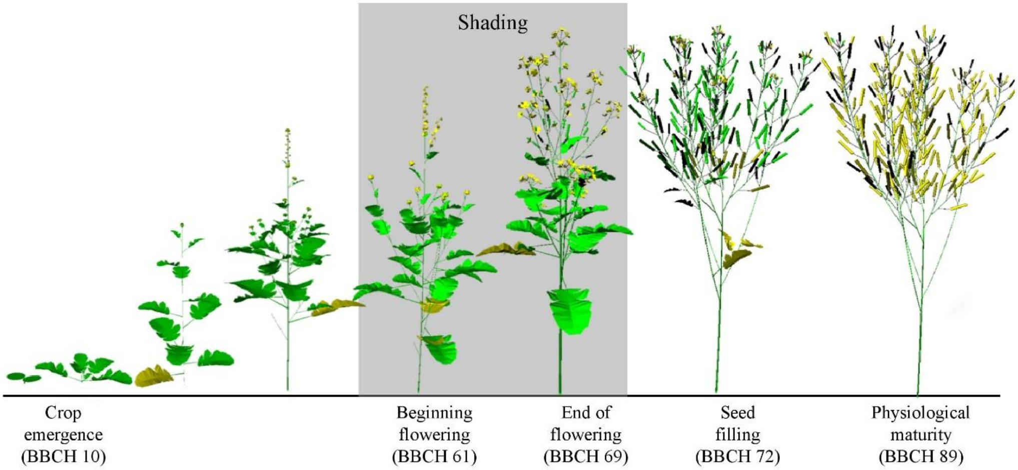

Fig. 4.1. Schematic diagram of oilseed rape developmental stages and moment when shading was established.

In Experiment 1 (Exp. 1) plants were sown on September 4, 2014 at two plant densities (40 and 80 plants m^{-2}) and two source-sink treatments (full sunlight and plots shaded by a black net intercepting 70% of the incident PAR between beginning of flowering [BBCH 61] and end of flowering [BBCH 69], i.e., during 19 days). The treatments were arranged in a split–plot block design with three replicates, where plant population was assigned to main plots and source-sink treatments to sub-plots. Their effects were tested on oilseed rape plants sown in plots of 2 × 4 m size of 11 rows at 0.175 m apart.

Experiment 2 (Exp. 2) was sown on September 28th 2015 at two plant densities (30 and 45 plants m^{-2}) and two source-sink treatments (the same as in Exp. 1 except for the duration of shading, which was 28 days). The plot size and experimental design were the same as in Exp. 1. Since plant densities used in Exp. 1 (40 and 80 plants m^{-2}) did not affect seed yield, for Exp. 2 lower densities were used (30 and 45 plants m^{-2}).

4.2.2. Experimental management and weather data

In both experiments plots were fertilized at sowing with phosphorus (150 kg ha^{-1} of $Ca(H_2PO_4)_2$) and potassium (100 kg ha^{-1} of $K_2SO_4.MgSO_4$). Nitrogen fertilization (50% NO_3 and 50% NH_4) was split into two applications: 80 kg N ha^{-1} five days after plants emerged (BBCH 10) and 80 kg N ha^{-1} when the fifth internode was expanded (BBCH 35). Plots were irrigated weekly to complement rainfall and to avoid water shortages until harvest in both growing seasons. To keep plants free of biotic stresses, weeds were periodically removed by hand, while diseases and insects were prevented with fungicides or insecticides at the rates recommended by their manufacturers. In Exps. 1 and 2 the shading nets were kept open on the south side to facilitate free air circulation and access for pollinators.

Weather data (maximum and minimum air temperatures and incident solar radiation) were recorded at 30 minutes intervals from sowing until harvest at the meteorological station of Universidad Austral de Chile (https://centroccbb.cl/clima/indexData.php) located at 5 km of the experiment. Air temperature under the nets was on average less than 1 °C lower than in controls as was shown in previous experiments at the Estación Experimental Agropecuaria Austral (Sandaña et al., 2009).

Table 4.1. *List of abbreviations, their definitions and units.*

Symbol	Variable name	Definition	Units
BBCH	Biologische Bundesanstalt, Bundessortenamt and Chemical Industry	Phenological scale for mono-and dicotyledonous plants	-
D40, D80	Plant density	Number of plants in 1 square meter, during Exp. 1	plants m^{-2}
D30, D45	Plant density	Number of plants in 1 square meter, during Exp. 2	plants m^{-2}
IPAR	PAR intercepted by the canopy	Difference between PAR_{inc} and PAR_{trans}	µmol m^{-2} s^{-1}
HI	Harvest index	Ratio between seed yield and biomass	%
PAR	Photosynthetically active radiation	Spectral range of solar radiation between 400 - 700 nm	µmol m^{-2} s^{-1}
PAR_{inc}	PAR incoming	Incident PAR above the canopy	µmol m^{-2} s^{-1}
PAR_{trans}	PAR transmitted	PAR_{inc} that is not intercepted by the canopy	µmol m^{-2} s^{-1}
R/FR	Red-far red ratio	Red = 680 nm; far red = 730 nm	-
T_{mean}	Mean temperature	Average daily air temperature	°C
T_{base}	Base temperature	Lowest temperature where metabolic processes result in a net substance gain in aboveground biomass	°C
TT	Thermal time	Accumulation of daily mean temperature above a base temperature	°C day

4.2.3. *Light environment at flowering stage*

Seven days after the beginning of flowering (BBCH 65), light intercepted by the canopy (*IPAR*) was calculated as the difference between incident (PAR_{inc}) and transmitted (PAR_{trans}) light recorded by a 1 m long LI-191 R quantum line sensor (LI-COR Inc., Lincoln, NE, USA). To estimate PAR_{inc}, the sensor was held 10 cm above the canopy, while for PAR_{trans} the line sensor was held at 100 cm of height (corresponding at the bottom of the main raceme), at 75 cm of height (corresponding at the bottom of the first branch), 50 cm (corresponding at the bottom of the 6th branch), and at ground level. At the same stage, and similar to PAR, measurements of R:FR were performed 10 cm above the canopy, at 100, 75, 50 cm of plant height and at ground level, using the Skye SKR 110 Red/Far Red sensor (Skye Instruments Ltd, Llandrindod Wells, UK). Both PAR and R:FR measurements were made at noon during sunny days. R:FR measurements were done with the sensor facing parallel to the soil surface oriented to the north, south, east and west, which allowed the light scattered by neighbouring plants to reach the sensor. The average of the four values was used for further analysis.

4.2.4. *Plant measurements*

Seven days after plant emergence, when cotyledons were completely unfolded (BBCH 10), five representative plants were randomly selected and labelled for each plot to evaluate crop developmental according to the BBCH phenological scale for oilseed rape (Meier, 2001). The time was expressed in thermal time units (*TT*) calculated by summing daily mean temperatures (T_{mean}) using a base temperature (T_{base}) of 0 °C for spring oilseed rape (Kirkegaard et al., 2012):

$$TT = \sum_{i}^{n} (T_{\mathrm{mean\ i}} - T_{\mathrm{base}}) \qquad (\mathrm{Eq.\,4.1})$$

When the labelled plants reached maturity, defined as the moment when nearly all the seeds inside the siliques were dark and hard (BBCH 89), they were sampled separately to evaluate individual plant fitness. These plants were dried at 65 °C for 48 h, biomass and the number of siliques per plant were recorded; afterwards, each silique was manually threshed to obtain seed yield, biomass and harvest index (HI). Seed yield per silique was evaluated in the same labelled plants by recording seed number per silique and the single-seed weight in siliques positioned at both the top and bottom of the main raceme and on the most basal branch (sixth branch counted from the top). Fresh weight and dry weight (after oven drying at 65 °C for 48 h) were measured, in all seeds contained in individual siliques positioned on the main raceme. Siliques positioned on the first and sixth branches were sampled weekly and divided into the number of seeds by silique to obtain fresh and dry weight per seed. Seed water content was estimated as the difference between fresh and dry seed weight. Thus, the relationship between dry seed weight and seed water content was assessed from the beginning of seed filling to maturity in seeds positioned at different places on the plants, in order to evaluate the potential effects of shading on the growth rate of seeds and/or on the duration of the seed filling phase.

At harvesting time all the plants in 1 m^2 were sampled at each plot, threshed and weighed after oven drying for 48 hours at 65 °C to determine biomass and seed yield per area with an analytical balance (Mettler, Toledo XP205DR, Greifensee, Switzerland). Seed number per square meter was counted by using a *Contador* seed counter (Pfeuffer GmbH, Kitzengen, Germany), and average seed weight per sample was calculated as the average weight of three random subsets of 1000 seeds each. From these samples, oil and protein concentrations were also measured. Seeds were ground and fatty acids were extracted with hexane based on the Soxhlet extraction method (de Castro and Garcia-Ayuso, 1998). On the defatted flour, protein concentration was measured by the Bradford protein assay (Jones et al., 1989), and results of both oil and protein were expressed on dry matter basis.

In seed crops, the seed weight dynamics are generally divided into three phases. It begins with a period of active cell division known as the "lag phase" with no significant gains in seed weight, followed by a second phase of "effective period of seed filling" characterized by a rapid gain in seed weight and a "plateau" where the seed stops its growth (Bewley and Black, 1994). The current study focused on the last two phases - the "effective period of seed filling" and the "plateau"- by fitting a split-line model to the data (Murray and Wood, 2016):

$$S1 = (x - PMx) \times (x < PMx)$$
$$S2 = (x - PMx) \times (x > PMx) \qquad \text{(Eq. 4.2)}$$

where *S1* (Slope 1) corresponds to the "seed growth rate" during the effective period of seed filling, and *S2* (Slope 2) is assumed to be the "plateau" (with a slope equal to zero). x is the thermal time after flowering and *PMx* is "physiological maturity" or the moment when the maximum seed weight is reached. This breakpoint is determined by minimizing the sum of squares (Payne et al., 2011).

Physiological maturity coincides with the moment when seeds reach 36% of moisture (Elias and Copeland, 2001), since below this threshold seeds continue losing moisture but the final seed dry weight does not change significantly. In the current study, physiological maturity was determined by fitting one linear model per group (treatment) to the relationship between seed water content and thermal time after flowering:

$$y = ax + b \qquad \text{(Eq. 4.3)}$$

where y is yield (per silique, plant or per unit area), a is the rate of yield change, x is the yield component evaluated and the intercept b corresponds to the yield (per silique, plant or per unit area) when the yield component is equal to zero.

4.2.5. Statistical analysis

Statistical differences in seed yield, quality and its associated traits in response to the plant density and source-sink ratio were tested by two-way ANOVA using GENSTAT v. 17 (Payne et al., 2011), and differences between treatments were considered statistically significant at a probability level of 5%. The following model was used to test differences among treatments:

$$Y_{ijk} = \mu + D_i + S_j + DS_{ij} + \varepsilon_{ijk} \qquad \text{(Eq. 4.4)}$$

where Y_{ijk} is the evaluated variable, μ is the mean of the observations and the effects of the treatments are represented as follows: D_i is the main effect of plant density, S_j is the main effect of shading, DS_{ij} is the interaction between plant density and shading, plus a residual ε_{ijk}, which represents the random variation. Where significant effects were found, differences among means were evaluated using Fisher's LSD test, while contrasts between treatment pairs (i.e., comparisons between high vs. low plant density or with vs. without shading) were assessed using a two-sample t-test. When differences in seed weight were observed, seed growth rate and the duration of the seed filling phase were evaluated (only for Exp. 2).

A slope difference test was performed to evaluate the rate of seed moisture loss in response to the treatments using GENSTAT v. 17. The probability of significance (*F. pr*) and the correlation coefficient (*r*) were used to evaluate the goodness of fit, which measures the strength and direction of a relationship between two variables (Payne et al., 2011).

4.3. Results

4.3.1. Crop phenology and weather conditions

The phenological stages of flowering and beginning of silique filling were not affected by the treatments (data not shown). Plants across the treatments reached the beginning of flowering at 687 and 514 °C day after emergence, in Exp. 1 and 2, respectively. The shorter phase up to the beginning of flowering in Exp. 2 could be due to the delay in sowing date with longer photoperiod and higher minimum and maximum temperatures compared to Exp. 1, while global radiation was similar in both experiments (Fig. 4.2).

In the shading treatment, the PAR_{inc} was reduced by 70% (Fig. A4.1), and the black net used for shading did not affect the red:far red ratio of the light (Fig. A4.2), leading to no undesirable photomorphogenic effects. In Exp. 1 the real plant densities were 40 ± 0.3 and 80 ± 4.8 plants m^{-2} in the control, and 40 ± 1.2 and 80 ± 4 plants m^{-2} under shading. In Exp. 2 the real densities were 3.0 ± 5.8 and 45 ± 5.2 plants m^{-2} in the control, and 40 ± 3.5 and 80 ± 4.7 plants m^{-2} under shading.

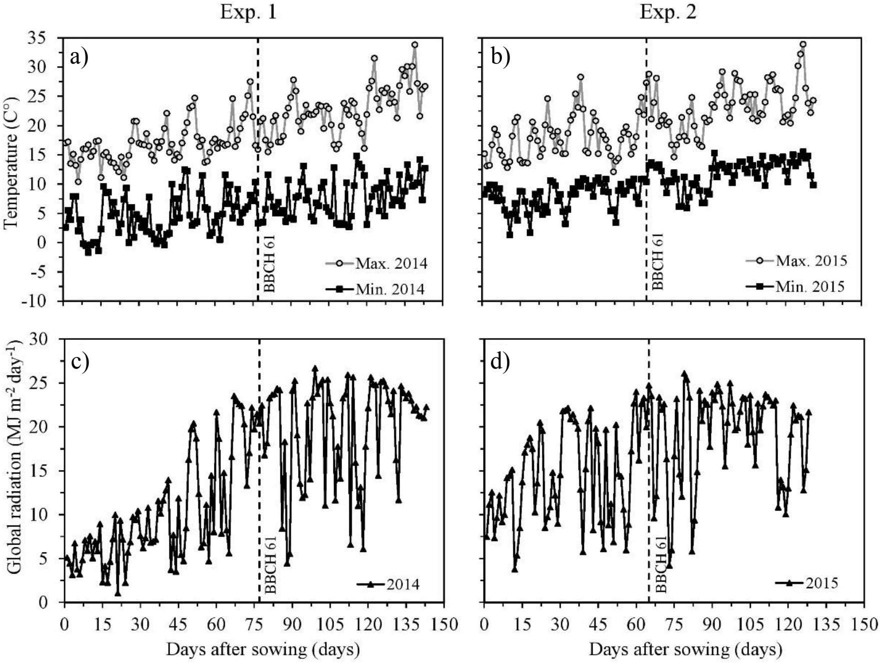

Fig. 4.2. Daily temperatures and global radiation between sowing and harvest during Experiment 1 (a and c) and Experiment 2 (b and d). Dashed line represents the beginning of flowering stage (BBCH 61).

4.3.2. Seed yield and associated physiological traits

Seed yield per area (Table 4.2) was not affected ($P>0.05$) by either plant density or shading in Exp. 1. This was a consequence of the compensation between plant number and yield per plant as the latter was 30 and 50% higher ($P<0.05$) at D40 than at D80 under control and shading, respectively (Table 4.2). Increases of siliques (by 39 and 69% in control and shading, respectively) (Table 4.2) and biomass per plant (by 29 and 36% in control and shading, respectively) (Table 4.3) were also recorded in D40 relative to D80. Biomass partitioning towards the seeds varied in a narrow range across treatments in Exp. 1 (23.5-26.9%), showing interaction ($P<0.05$) between plant population and shading (Table 4.3). The results observed in Exp. 2 were less conclusive than in Exp. 1, although seed yield per area was not different ($P<0.05$) among treatments in Exp. 2 (Table 4.2). Seed yield per plant showed a considerable interaction ($P=0.066$) between plant population and shading, high standard errors (Table 4.2) and a coefficient of variation of 15.5%. This suggests higher variability in Exp. 2 than in Exp. 1, likely because the shading period was longer in this experiment.

Table 4.2. Seed yield per area and per plant, siliques per area and per plant, seed number per area, and thousand seed weight in response to plant density and shading

Treatments		Seed yield per area (g m^{-2})	Seed yield per plant (g per plant)	Seed number per area (10^3 m^{-2})	Thousand seed weight (g)	Siliques per area (siliques m^{-2})	Siliques per plant (siliques per plant)
				Experiment 1			
D40	Control	302.2 ± 3.3	6.8 ± 0.3	91.4 ± 2.7	3.3 ± 0.1	6480 ± 401	148.3 ± 8.1
	Shading	286.6 ± 17.0	7.1 ± 0.8	55.0 ± 2.6	5.2 ± 0.2	5899 ± 859	135.5 ± 17.6
D80	Control	299.6 ± 20.1	5.2 ± 0.4	90.3 ± 6.0	3.3 ± 0.0	7766 ± 583	106.9 ± 11.1
	Shading	266.8 ± 53.6	4.7 ± 0.6	50.1 ± 9.8	5.3 ± 0.0	5844 ± 841	80.4 ± 12.5
	D	NS	*	NS	NS	NS	*
	S	NS	NS	***	***	NS	NS
	D × S	NS	NS	NS	NS	NS	NS
				Experiment 2			
D30	Control	223.3 ± 36.9	9.5 ± 1.5	80.0 ± 4.8	2.8 ± 0.3	4762 ± 751	184.6 ± 24.7
	Shading	208.3 ± 36.6	8.0 ± 1.8	46.5 ± 5.3	4.4 ± 0.4	4099 ± 1292	134.6 ± 36.7
D45	Control	245.7 ± 47.2	13.1 ± 3.2	81.3 ± 16	3.0 ± 0.1	9078 ± 1880	216.7 ± 42.6
	Shading	152.7 ± 9.9	6.8 ± 1.3	34.7 ± 1.3	4.4 ± 0.1	4836 ± 756	107.4 ± 15.4
	D	NS	NS	NS	NS	NS	NS
	S	NS	**	**	**	*	**
	D × S	NS	*	NS	NS	NS	NS

Asterisks indicate significant differences between treatments at *$P<0.05$, **$P<0.01$ and ***$P<0.001$, while NS = not significant ($P> 0.05$), according to ANOVA.

D = Plant density

S = Shading treatment

4.3.3. Seed yield components

Seed number per area and thousand seed weight were affected by shading in both experiments, whereas plant population showed no effect on them (Table 4.2). In Exp. 1 seed number per area was decreased by shading between 40 and 45% under D40 and D80, respectively, while the number of siliques per area and per plant were not affected ($P>0.05$). In Exp. 2 reductions in seed number per area in response to shading reached 57% under D45 (Table 4.2) and were accompanied by reductions in the number of siliques per plant and per area ($P<0.05$). Remarkably, and in contrast to the assumption considering seed weight as a highly conserved trait, thousand seed weight of shaded plots increased ($P<0.05$) by 58 and 61% relative to the control in Exp. 1 (Fig. A4.4), and by 47 and 59% ($P<0.05$) over control plants in Exp. 2.

To better understand the effect of treatments, seed yield, seed number and single-seed weight were also evaluated at the plant level for different branches. Seed yield of the main raceme was higher than that of the sixth branch. Siliques set at the bottom of the main raceme showed higher ($P<0.05$) yield per silique than in the top position in both Exp. 1 and 2 (Figs. 4.3a and 4.4a, respectively). Interestingly, higher yield per silique in shaded plants was mainly due to heavier ($P<0.05$) seeds showing increases of seed weight between 11 and 105% over control plants in Exp. 1 (Fig. 4.3c) and between 26 and 134% in Exp. 2 (Fig. 4.4c). A visual example of the seed weight plasticity is shown in Fig. A4.4. By contrast, the effect of shading on the number of seeds per silique was less clear, showing reductions between 12 and 18% in siliques set at the bottom of the main raceme in both experiments, with no effect ($P>0.05$) observed at other positions of the plants (Figs 4.3b and 4.4b).

Table 4.3. Biomass per plant, harvest index, oil and protein concentrations in response to plant density and shading

Treatments		Biomass per plant (g per plant)	Harvest index (%)	Seed oil concentration (%)	Seed protein concentration (%)
			Experiment 1		
D40	Control	26.9 ± 1.3	25.3 ± 0.2	38.1 ± 0.6	32.9 ± 0.1
	Shading	26.6 ± 3.9	26.9 ± 0.8	38.7 ± 1.8	31.9 ± 1.9
D80	Control	20.9 ± 1.3	24.6 ± 0.6	39.2 ± 0.2	32.4 ± 1.3
	Shading	19.6 ± 2.3	23.5 ± 0.7	38.2 ± 0.8	34.5 ± 0.3
	D	*	**	NS	NS
	S	NS	NS	NS	NS
	D × S	NS	*	NS	NS
			Experiment 2		
D30	Control	29.6 ± 4.3	32.2 ± 1.2	48.5 ± 0.3	33.2 ± 0.2
	Shading	26.3 ± 6.4	31.6 ± 2.2	46.3 ± 1.3	35.8 ± 0.7
D45	Control	42.2 ± 12.6	32.7 ± 1.8	49.0 ± 0.1	37.2 ± 0.7
	Shading	19.9 ± 2.8	33.7 ± 2.5	46.5 ± 0.7	35.5 ± 1.9
	D	NS	NS	NS	NS
	S	**	NS	**	NS
	D × S	NS	NS	NS	NS

Asterisks indicate significant differences between treatments at *$P<0.05$, **$P<0.01$ and ***$P<0.001$, while NS = not significant ($P> 0.05$), according to ANOVA.

D = Plant density

S = Shading treatment

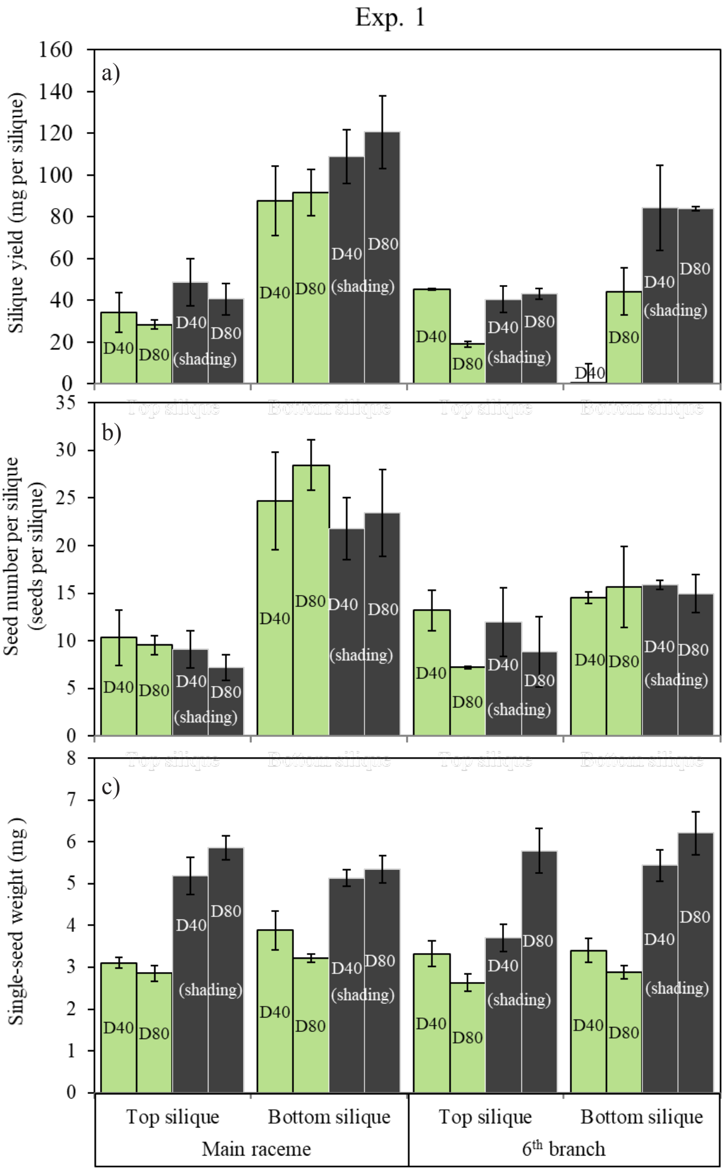

Fig. 4.3. a) Silique yield, b) seed number per silique and c) single-seed weight for siliques positioned on the main raceme and at the sixth branch in Experiment 1.Vertical bars indicate standard error of means.

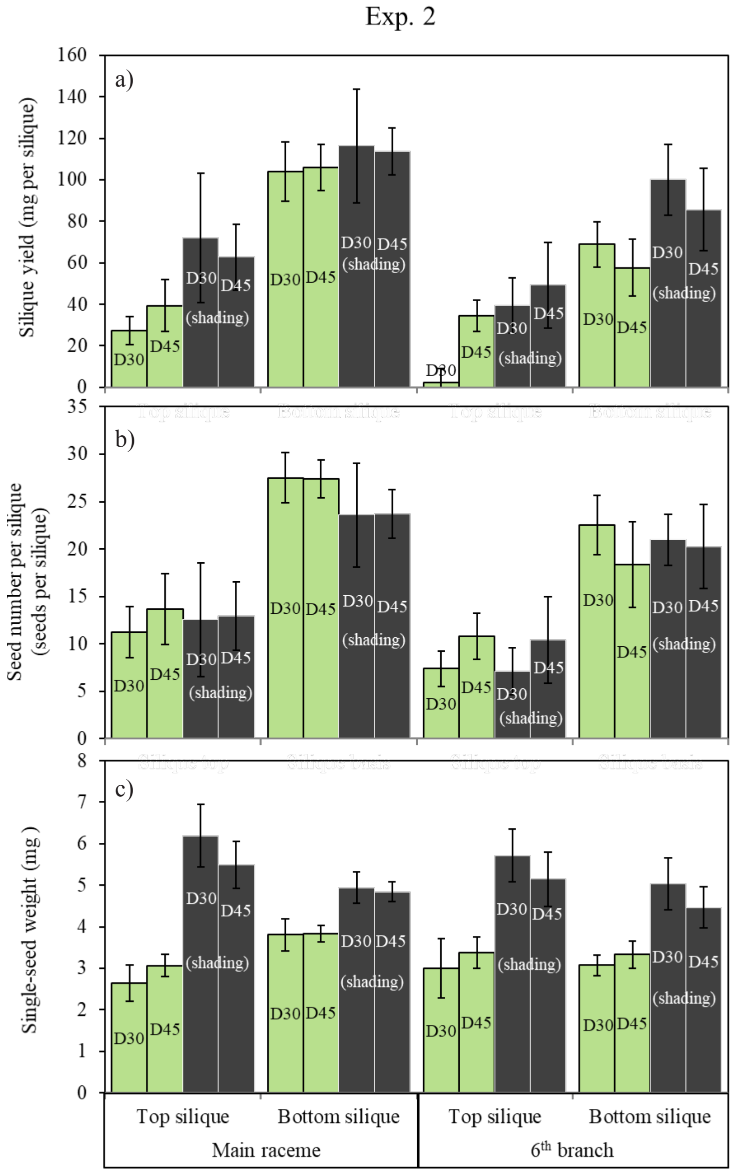

Fig. 4.4. a) Silique yield, b) seed number per silique and c) single-seed weight for siliques positioned on the main raceme and at the sixth branch in Experiment 2. Vertical bars indicate standard error of means.

4.3.4. *Rate and duration of seed filling*

Seed filling rate and duration, measured only in Exp. 2 were affected by shading ($P<0.05$), but not by plant density ($P>0.05$). On the main raceme, seed filling rate was higher (Fig. 4.5a) under shading than in the control plants (i.e. 9 and 92% for D30 and D45, respectively), while on the sixth branch increases of 15 and 47% were recorded for D30 and D45, respectively (Fig. 4.5b). The seed filling period was also increased by shading in comparison with the control, but at a lower extent than seed filling rate as it was found on the main raceme (i.e. 6 and 4% for D30 and D45, respectively) and on the sixth branch (i.e., 4 and 23% for D30 and D45, respectively). Therefore, higher seed weight reached by shaded plants was the consequence of both higher seed filling rate and longer seed filling period, while the magnitude of such effects depended on the position of the silique on the plant. The relationship between seed moisture and seed weight on the main raceme was an exponential decay (Fig. 4.5c). For seeds positioned on the sixth branch, only control plants showed this trend, because a linear relationship fitted better than the curvilinear one for shaded seeds under D30 and D45 (Fig. 4.5d).

4.3.5. *Oil and protein concentration*

In Exp. 1 oil concentration (Table 4.3) did not vary ($P>0.05$) in response to plant density or shading. However, in Exp. 2 oil concentration of shaded plants was significantly ($P<0.05$) lower than under full sunlight by 5% (Table 4.3), but this did not match the reduction by 70% of incoming PAR during flowering. The protein concentration was highly conserved across the treatments ($P>0.05$) and between Exps. 1 and 2 (Table 4.3).

4.4. Discussion

The central objective of our study was to evaluate the plasticity of seed number, thousand seed weight, oil and protein concentrations of oilseed rape in response to the availability of resources both per plant and at a specific phenological phase by manipulating plant density and source-sink ratio at flowering, respectively.

4.4.1. *Seed yield and associated traits in response to plant density*

Seed yield per area did not vary when plants grew at 40 and 80 plants m^{-2} (Exp. 1), or at 30 and 45 plants m^{-2} (Exp. 2), showing a remarkable compensatory capacity of oilseed rape. The stability of yield was observed when the plant density was reduced by a factor two, particularly when the reduced plant population was uniformly distributed (Angadi et al., 2003). At low plant density, oilseed rape plants had higher biomass, number of branches, siliques and ultimately higher seed number per plant (Momoh and Zhou, 2001). In contrast, thousand seed weight was highly conserved and did not vary in response to plant density, which is in line with previous studies in oilseed rape (Angadi et al., 2003; Momoh and Zhou, 2001).

Decreases in seed number per plant are associated with the decline of the number of siliques per branch and seed number per silique in lateral branches rather than on the main raceme (Zhang et al., 2012). In our study, siliques positioned at the bottom of the main raceme showed higher yield than those at other locations in the plant, while within a branch siliques positioned at the bottom of each branch showed a higher yield than those in the top.

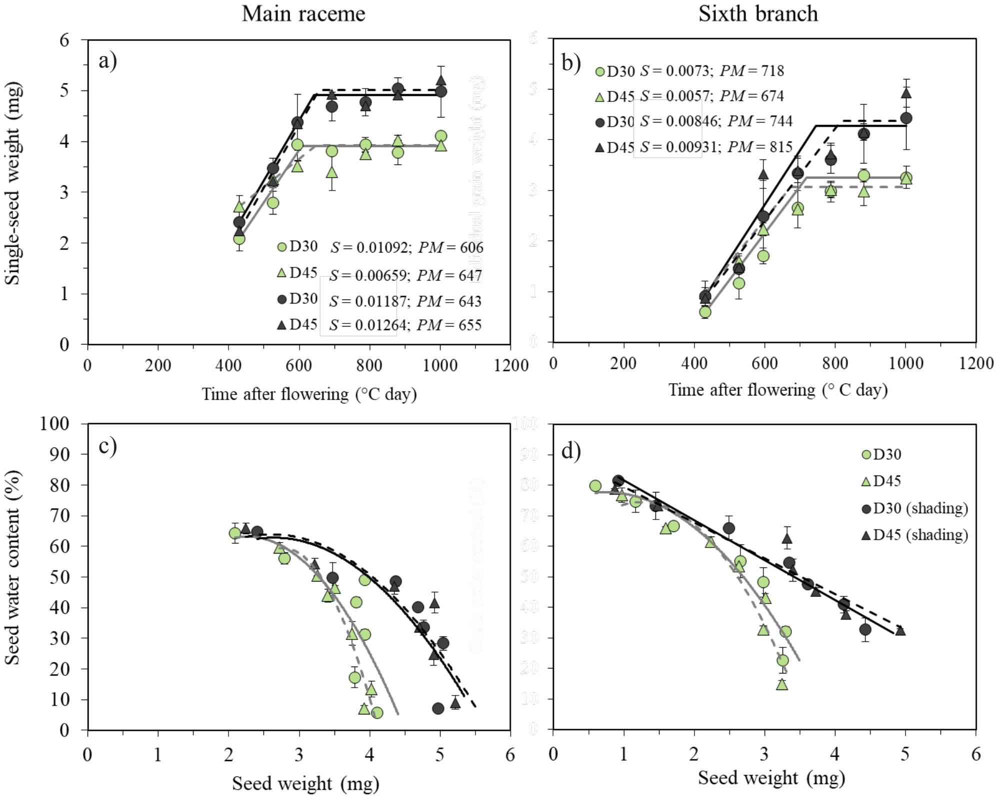

Fig. 4.5. Single-seed weight and relationship between seed moisture and seed weight for seeds positioned on the main raceme (a and c, respectively) and on the sixth branch (b and d, respectively) during seed filling of the 2015-2016 season. Fitted grey and black lines represent the trends of control and shading, respectively, while solid and dashed lines represent D30 and D45, respectively. S1 is the seed filling rate and PM is physiological maturity.

4.4.2. *Seed yield and associated traits in response to shading*

Shading at flowering did not affect seed yield per unit of area in our experiments. Although the stability of seed yield was less clear in D40 of Exp. 2, likely because the shading period was extended for 10 more days affecting also seed weight, the resilience of seed yield shown by oilseed rape is in contrast with the reported responses of other seed crop species (Fischer, 1985; Sandaña and Calderini, 2012). In Exp. 1, there was a significant interaction between plant density and shading for traits such as biomass and harvest index, whereas in Exp. 2, there was a reduction in biomass, but the harvest index was not affected. Similar experiments performed in pea and lupine (semi-determinate crops like rapeseed) showed that biomass and harvest index were reduced by shading over 15 days after flowering (Sandaña and Calderini, 2012). As a consequence, it is hypothesized that reductions in intercepted *PAR* during flowering reduced the crop growth rate of oilseed rape and then the total amount of assimilates and the fraction of assimilates partitioned towards the reproductive organs as has been observed in wheat (Sandaña and Pinochet, 2011).

Our results showed that shading at flowering reduced the seed number per area. Since oilseed rape branches expand basipetally, and the flowers bloom acropetally along the branch (Wang et al., 2011), beginning of flowering, flower abortion, silique set and beginning of seed filling overlap among flowers/siliques positioned in different parts of the canopy. Thus, the age and position of the flowers/siliques in the canopy determine their access to assimilates, and subsequent number of siliques per plant and seed number per silique (Wang et al., 2011). Reductions in seed number per area in response to shading in our experiment were due to reductions in both the number of seeds per silique, and the number of siliques per area (see Fig. A4.6). Surprisingly, the negative effect of shading on seed number per area was fully compensated by an increase in thousand seed weight ranging from 47 to 61%. This increase was largely a result of both a higher seed filling rate and a longer seed filling duration across all seed positions in the canopy (Fig. 4.4). In crops like wheat, source-sink ratio manipulations around flowering are expected to have higher impact on seed weight than in later stages, because ovary size is increasing and endosperm cells are divided at pre- and post-anthesis, respectively (Lizana et al., 2010). Thus, increases in seed weight in response to shading at flowering have only been observed in sorghum (*Sorghum bicolor* L.), where seed weight increased by 31% (Kiniry, 1988), but in that case seed weight plasticity did not fully compensate for reductions in seed number. In previous studies carried out on oilseed rape, the source-sink ratio was manipulated around flowering in three different experiments performed in growth chambers (Tayo and Morgan, 1979). The source was reduced by defoliating 100% of the leaves one week after flowering, or reducing incoming PAR by 85% or 100%. In two of the experiments, there were reductions in seed weight by 6-22%, in response to the treatments, while in the third experiment seeds were 10% heavier and 7% lighter depending on their position in the canopy, i.e. at the bottom or on the top of the canopy, respectively. On the other hand, the increase of the source-sink ratio by silique removal at the same stage did not affect seed weight (Tayo and Morgan, 1979). Thus, the magnitude of plasticity in seed weight found in our study on oilseed rape has never been reported before in literature for this or another grain crop (Kiniry, 1988; Sadras, 2007; Tayo and Morgan, 1979).

4.4.3. Oil and protein concentrations are conservative traits

Oil concentration was very stable in response to both plant density and shading. Previous studies have shown that increasing inter-plant competition reduces oil concentration (Momoh et al., 2004), while others have found a positive effect on oil concentration (Zhang et al., 2012), or no effect at all (Leach et al., 1999). In the present study, plant density did not affect oil concentration, supporting the suggestion that it is a stable trait.

The synthesis of fatty acids takes place during the seed filling period (Diepenbrock and Geisler, 1979), with a peak between 15 and 35 days after flowering (Tzen et al., 1993), a period during which the fatty acid synthesis is highly sensitive to reductions in PAR_{inc} (Rondanini et al., 2014). Thus, it could be hypothesized that source-sink manipulations during flowering would not affect oil concentration. This hypothesis was validated in Exp. 1, however, reductions in oil concentration of around 4% have been observed when shading partially overlapped the seed filling period (Fortescue and Turner, 2007). These results could explain why oil concentration was decreased by 5% in Exp. 2, when shading was extended until the beginning of seed filling. Protein concentration was the most conserved trait in both experiments, showing no trade-off with oil concentration. Additionally oil and protein production per area, the main target for oilseed growers and breeders, were not affected by the treatments (Fig. A4.5).

In summary, oilseed rape can adjust its potential number and size of resource-demanding sinks based on the assimilates produced at different phases. During the vegetative stage, the environmental conditions modify plant architecture and phenology (Noquet et al., 2004), while at early flowering the plants can adjust the number of siliques per plant and seeds per silique according the source-sink ratio (Pinet et al., 2015). Our results demonstrated that plasticity of oilseed rape works even at the end of flowering by altering the potential seed weight, while oil and protein concentrations remained highly stable.

4.4.4. Implications of findings and conclusions

The hypothesis that seed number is highly responsive to the assimilate availability during flowering, while seed weight is almost insensitive has been confirmed in several crops (Sadras, 2007) and assumed for oilseed rape. Thus, seed number has been proposed as a candidate target trait to continue increasing seed yield in this crop (Diepenbrock, 2000; Gomez and Miralles, 2011). Our results demonstrated that, in contrast to other grain crops, plasticity of single-seed weight in oilseed rape was unexpectedly high, fully compensating for reductions in seed number under assimilate constraints at flowering. We also observed that factors that increase single-seed weight at flowering had little impact on seed oil and protein concentrations. Therefore, we propose that heavier seeds are a potential strategy to improve seed yield without negative effects on quality traits. To achieve this complex objective, physiological and genetic bases of the higher potential seed weight involved in the current study should be further elucidated. For instance, future research should investigate whether the expression of heavier seeds was triggered by the reduction of assimilates at flowering or by the abrupt increase in flux of assimilates towards the seeds, when the shaded plants received again 100% of the incoming PAR. In addition, yield progress by increasing potential seed weight will only be possible if there are no trade-offs between yield components. For this purpose, quantitative trait loci regulating independently seed number and weight should be identified, after which the use of Marker-Assisted Selection (MAS) breeding could be a promising tool to increase seed yield, as proposed for maize (Chen et al., 2016).

Finally, larger seeds can also offer agronomic advantages. The use of larger seeds at sowing time improves the seedling establishment, by reducing the period between sowing and emergence, increasing the early vigour, biomass (Harker et al., 2015) and *PAR* interception at flowering (Mendham et al., 1981). In marginal regions where rainfall are a constraint for crop establishment, larger seeds would allow deeper sowing in soil layers with higher moisture, improving crop establishment and early vigour (Brill et al., 2016). In addition, larger seeds increase the tolerance to pests such as flea beetles (*Phyllotreta spp.*) (Elliott et al., 2008). All these factors can contribute to reducing the use of agrochemicals for weed and insect control in farming systems. Thus, improving the knowledge about the impact of decreased source-sink ratio on seed yield components and their interactions found in the present study is critical for both plant breeding and agronomical managements of oilseed rape.

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Supplementary Material

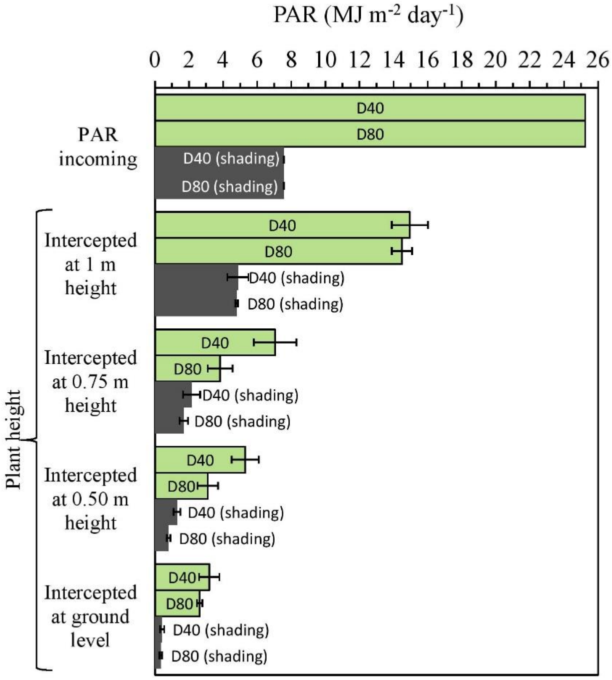

Fig. A4.1. Photosynthetically active radiation above the canopy (at 160 cm), at 1 m, 0.75 m, 0.5 m of plant height and at ground level at BBCH 65 in response to plant density and shading. Bars indicate standard error of means.

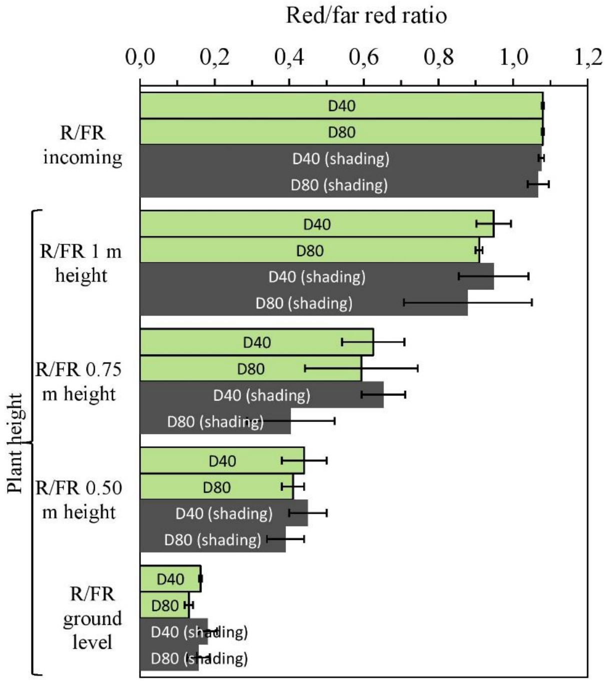

Fig. A4.2. Red:far red ratio, above the canopy (at 160 cm), at 1 m, 0.75 m, 0.5 m of plant height and at ground level at BBCH 65 in response to plant density and shading. Bars indicate standard error of means.

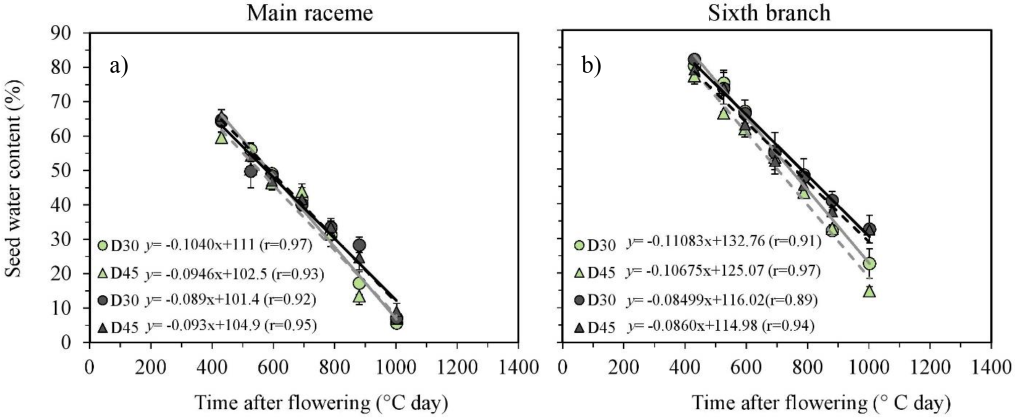

Fig. A4.3. Seed moisture for seeds positioned on the main raceme (a) and on the sixth branch (b), during seed filling of the 2015-2016 season. Fitted grey and black lines represent the trend for control and shading, respectively, while solid and dashed lines represent D30 and D45, respectively.

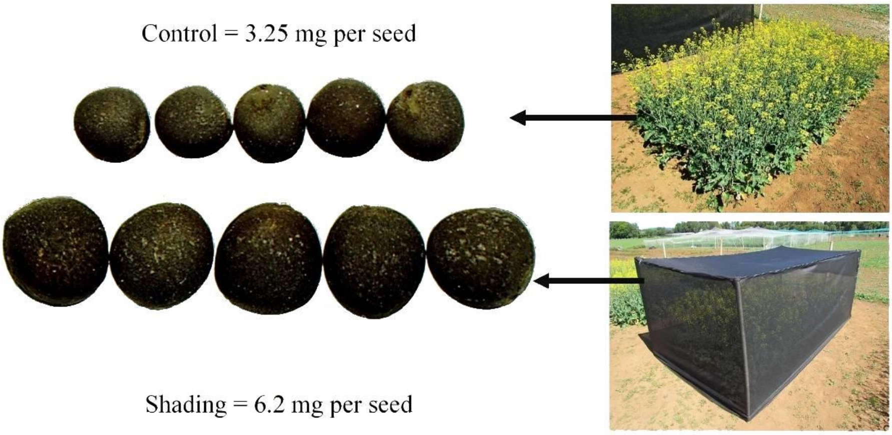

Fig. A4.4. Seed weight of oilseed rape under full sunlight (control) and shading during the 2014-2015 season (Exp. 1)

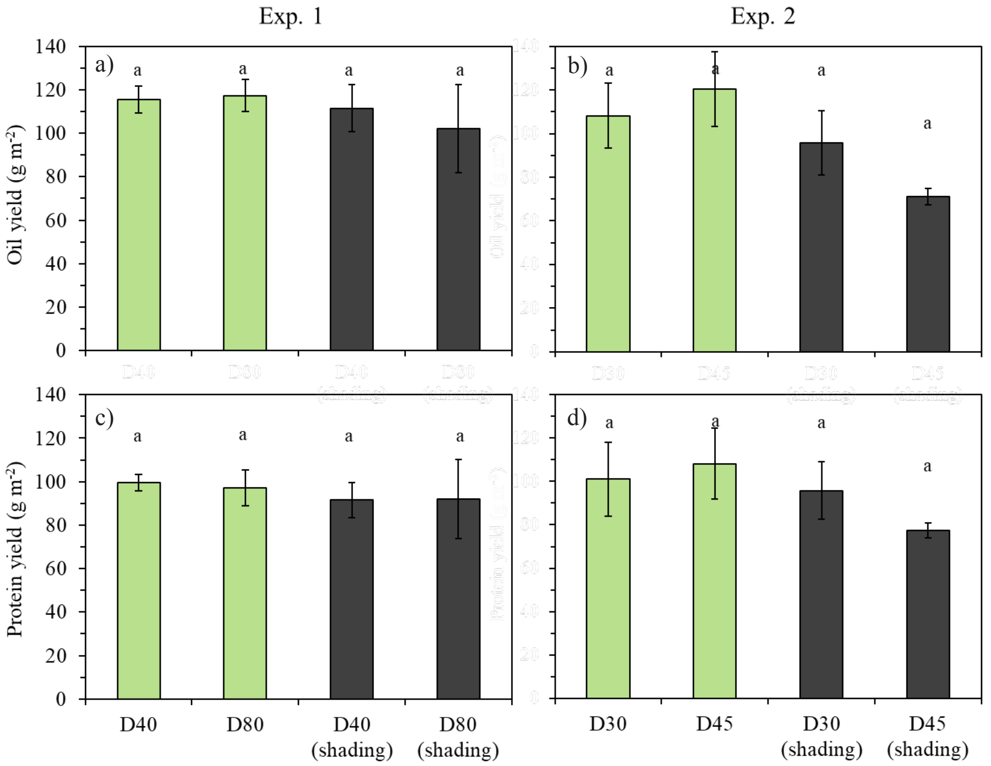

Fig. A4.5. Oil and protein yields in Experiment 1 (a and c, respectively) and in Experiment 2 (b and d, respectively). Different letters indicate significant differences across treatments (P< 0.05) and vertical bars indicate standard errors of mean.

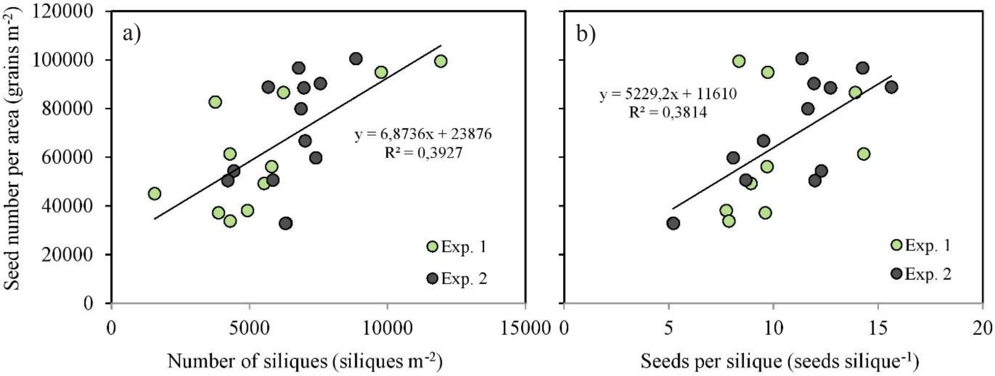

Fig. A4.6. Relationship between a) seed number and number of siliques per area and b) seed number and number of seeds per silique.

Chapter 5

Simulating plasticity of yield components of oilseed rape with functional-structural plant modelling

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Abstract

Seed yield of oilseed rape is determined by the plasticity of its associated traits, particularly at flowering, when plant carbon status is critical for determining the yield components. Since Functional-structural plant (FSP) modelling approach allows to evaluate plant organ response to simulated source/sink ratio, it is a helpful tool to address research questions concerning plant plasticity. The objectives of this study are: a) to identify the seed yield components that account for variation in seed yield and to quantify their sensitivity to resource availability, using datasets collected from three experiments and b) to evaluate the plastic response of seed yield to source/sink ratio manipulations at flowering using an FSP modelling approach.

The sensitivity of the seed yield components in response to plant density, N supply and source limitation at flowering showed the following the ranking: 1000-seed weight > number of siliques per plant > number of branches per plant > number of seeds per silique. Thousand seed weight was at the top of this ranking exclusively due to the 60 and 70% of heavier seeds observed in response to shading at flowering in two experiments. However, this trait did not respond to any other environmental factor. Therefore, the sensitivity of plant traits to environmental factors is not static and dependent on the crop, the type and timing of source limitation.

Different plant source/sink ratio were simulated by FSP modelling. The simulated shading caused a reduction in biomass, number of siliques per plant and number of seeds per silique, especially in plants growing at high density. The simulated removal of 50% of the siliques per plant, was partially compensated by an increase in yield per silique. These results suggest that the amount of assimilates available at flowering is the primary determinant of plant yield.

Additional keywords: assimilates, nitrogen, source/sink ratio, plant density, branching, silique

5.1. Introduction

The global production of oilseed rape, source of the third most consumed oil after soybean oil and palm oil, has increased reaching 71 million tonnes in 2017 (FAOSTAT, 2020). Since the expansion of cultivated area does not seem to be a large-scale sustainable strategy, there is agreement that increasing seed yield, by plant breeding and by optimizing agronomic practices, is the most feasible way to continue increasing crop production in the coming decades (Beddington, 2010; Hall and Richards, 2013). After the 1970s, the progress in productivity of oilseed rape was mostly achieved by increasing seed yield per unit area (Berry and Spink, 2006; Kirkegaard et al., 2016), where plant breeding and the intensification of agronomic practices, such as nitrogen (N) fertilizer input (Tilman et al., 2002) and optimization of sowing density (Hu et al., 2017), made an enormous contribution to the improvement of seed yield. However, during the last years the yield progress in countries such as the United States, the United Kingdom, Brazil, Finland, Sweden and the Czech Republic showed evidences of a levelling-off (Beddington, 2010; Peltonen-Sainio et al., 2007a; Rondanini et al., 2012), suggesting that new strategies to achieve further yield improvement should be designed and evaluated. One such strategy revolves around making use of plasticity in yield components.

Plant performance is the combined result of several physiological processes (White et al., 2015), modulated by environmental factors (Arnold et al., 2019; Valladares et al., 2007), which can be summarized by the source-sink paradigm. In general terms, the sources are the plant organs where net fixation of carbon dioxide occurs and the sinks are the organs where assimilates are stored or used (White et al., 2015). The assimilate allocation is driven by the relative sink strength, defined as the amount of assimilates needed to achieve potential growth relative to the sum of sink strengths of all growing organs in the plant (Heuvelink, 1996). In the case of oilseed rape, the leaves are sources of assimilates, stem and branches are sinks, while siliques and seeds are both sources and sinks at the same time. The seed yield in this crop is determined by the combined plasticity of following five components: number of plants per unit area, number of branches per plant, number of siliques per branch, number of seeds per silique and average seed weight (Diepenbrock, 2000). These yield components follow a “hierarchy”, term used by Bradshaw (1965) to describe the high plasticity of seed number compared with the stability of seed weight. Genotypes of cereals and legumes have been evaluated across several environments (Borras et al., 2004; Pereira et al., 1999; Sadras, 2007), and in response to variations in plant carbon status at flowering (Arisnabarreta and Miralles, 2008; Sandaña and

Calderini, 2012) and it was found that seed yield is mostly explained by seed number than by seed weight. Taking the hypothesis that seed number is a plastic trait, while seed weight is conserved and considering other yield components, Sadras and Slafer (2012) performed a meta-analysis a of published data for wheat, triticale, barley and rice, and ranked the plasticity of the yield components of these crops, in response to their sensitivity to environmental factors as follows: tiller number > inflorescence number ≈ seeds per inflorescence > seed weight. In oilseed rape, seed weight has also been considered as a highly stable trait (Peltonen-Sainio et al., 2007b). However, seed weight has shown higher plasticity than generally accepted for most grain crops, when incident photosynthetic active radiation (PAR) is reduced at flowering (Kirkegaard et al., 2018; Labra et al., 2017; Verdejo and Calderini, 2020; Zhang and Flottmann, 2018). Therefore, and in the light of previous results, the ranking of plasticity of yield components proposed by Sadras and Slafer (2012) for cereals, would be different in oilseed rape.

After flowering fertile ovules develop into seeds, becoming siliques into strong sinks for carbon assimilates produced by sources such as green leaves and the same siliques (Li et al., 2019; Wang et al., 2011). The number of siliques per plant and the yield per silique are mainly determined by the plant carbon status at flowering (Kirkegaard et al., 2018; Wang et al., 2016). At this stage, there is an overlap in flower growth, silique and seed setting, as well as stem, branch and leaf growth (Iglesias and Miralles, 2014). Thus, there is a strong competition for sources among several sinks after initial flowering and assessing the effect of the source-sink status on each yield component is not a complex objective. In field experiments where the plant source/sink ratio was reduced by reducing incident PAR by 70% during 19 days after from the starting of flowering on, seed yield per unit area, per plant and per silique were not affected (Chapter 4). In the same study, reductions in the number of siliques per plant and in the number of seeds per silique were fully compensated by the increased average seed weight. In other studies (Kirkegaard et al., 2018; Verdejo and Calderini, 2020; Zhang and Flottmann, 2018), in which similar source manipulations were performed at flowering, compensations were only partial, and seed yield per area was reduced.

Plant source/sink ratio can also be manipulated by reducing sink strength, but this type of manipulation has been less explored. Keiller and Morgan (1988) removed flowers positioned at the bottom of the main raceme, and they observed an increase in the number of smaller siliques in the main raceme and lateral branches, while the flowering period was also extended. Until today, little information is available on sink strength manipulation at flowering (without manipulating branching), due to operational difficulties to perform this manipulation in plastic crops like oilseed rape maintaining the proposed source-sink ratios.

Profiting from plant traits associated with high crop performance depends on the quantitative understanding of the extent to which sources or sinks limit plant growth during the plant life cycle (White et al., 2015), and the response of the yield components to agronomic practices. This requires an analytical approach integrating plant organ growth, its response to environmental factors, and a final upscaling to the level of biological organization relevant for agricultural research (i.e. yield per plant and per unit area). Crop growth models, such as LINTUL-BRASNAP (Habekotté, 1997a), CERES-RAPE (Gabrielle et al., 1998) and CECOL (Husson et al., 1998), simulate seed yield in oilseed rape without taking into consideration plant architecture and its interaction with physiological processes, fundamental characteristics to explore the stability of yield components in plastic crops like oilseed rape. Functional-structural plant (FSP) modelling approaches explicitly describe the development over time of the three-dimensional (3D) architecture of plants in a plant stand, as governed by physiological processes that, in turn, depend on environmental factors at the level of the plant organ, whole plant and plant stand (Evers et al., 2018; Vos et al., 2010). These characteristics make FSP modelling a suitable tool to address research questions concerning plant plasticity. The objectives of this study are: a) to identify the seed yield components that account for variation in seed yield and to quantify their sensitivity to resource availability, using datasets collected from three experiments and b) to evaluate the plastic response of seed yield to source/sink ratio manipulations at flowering using an FSP modelling approach.

5.2. Materials and methods

5.2.1. Experimental set-up and treatments

Spring oilseed rape cv. 'Solar CL' (NPZ-Lembke, Germany) was sown at Wageningen (51° 58' N., 5° 40' E.), the Netherlands, in 2013 (NL 2013), and at Valdivia (39° 47' S., 73° 14' W.), Chile, during the years 2014 (CL 2014) and 2015 (CL 2015). The experiment NL 2013 was carried out in an open-sided shelter under semi-controlled conditions, where plants were sown in containers (0.7 × 0.9 × 0.4 m). The treatments consisted of combinations of two contrasting rates of N supply: 50 (N50) and 150 (N150) kg ha^{-1} and two plant population densities: 50

(D50) and 150 (D150) plants m^{-2}. Details on the experimental set-up can be found in Chapter 2. Experiment CL 2014 was performed at field conditions at two plant densities (40 and 80 plants m^{-2}) and two source-sink treatments (full sunlight and plots shaded by a black net intercepting 70% of the incident PAR between beginning of flowering [BBCH 61] and end of flowering [BBCH 69], i.e., during 19 days). Experiment CL 2015 was also performed under field conditions, but at two other plant densities (30 and 45 plants m^{-2}) and two other source-sink treatments. The plot size of both experiments CL 2014 and CL 2015 was 2 × 4 m, while the treatments were arranged in a split–plot block design with three replicates, where plant population was assigned to main plots and source-sink treatments to sub-plots. The shading nets were kept open on the south side to facilitate free air circulation and access for pollinators. Further details of experimental setup of these two experiments can be found in Chapter 4.

5.2.2. Data collection

For all experiments, phenological growth stages in oilseed rape were recorded according to the BBCH phenological scale (Meier, 2001), while the time was expressed in thermal time units calculated using a base temperature of 0 °C for spring oilseed rape (Kirkegaard et al., 2012). After plant emergence, 30 representative plants per treatment were randomly selected and sampled at the following phenological stages: rosette (BBCH 30), flower buds visible but still closed or budding (BBCH 50), first flowers open (BBCH 60), 50% flowers on main raceme open, older petals falling (BBCH 65), 10% of siliques reached final size (BBCH 71), 50% of siliques reached final size (BBCH 75) and physiological maturity (BBCH 89). Dry matter of stems, branches, leaves, flowers and siliques was recorded after oven drying at 70 ºC for 48 hours. In Experiment NL 2013, leaf area (LI 3100; LI-COR, Lincoln, Nebraska, USA) and geometry of individual leaves were recorded, while leaf growth duration, flower growth duration and silique maturity were recorded twice a week by non-destructive measurements. Photosynthesis rate of green leaves and siliques was measured 7 days before and 27 days after flowering, respectively, with a Li-COR 6400XT Portable Photosynthesis System (LI-COR, Lincoln, Nebraska, USA), at saturated incident light on surface area and at a temperature of 25 °C.

In Experiments NL 2013, CL 2014 and CL 2015, five representative plants per container or plot were randomly selected and labelled 7 days after plant emergence, to be individually harvested at physiological maturity. At this stage, yield per plants, seed number per silique and average seed weight were evaluated from siliques positioned at both the top and the bottom of the main raceme, on the lateral branch and on the most basal branch. Yield components per plant were evaluated by recording the number of branches per plant, number of siliques per plant, number of seeds per plant and 1000-seed weight per plant on five individual plants located in the centre of each container (NL 2013) or five plants positioned in the centre of each plot (CL 2014 and CL 2015). The plants were dried at 65 °C for 48, the number of siliques per plant was recorded; afterwards, each silique was manually threshed to obtain seed number per silique, single-seed weight, seed yield per silique and per plant. Seed yield per silique was evaluated in the same labelled plants by recording the yield of individual siliques positioned at both the top and the bottom of the main raceme, on the lateral and on the most basal branch (sixth branch counted from the top). The number of seeds per silique corresponds to the number of seeds inside each evaluated silique, while the single-seed weight was calculated as the yield per silique divided by number of seeds per silique. Single-seed dry weight was measured after oven drying at 65 °C for 48 h. Also at physiological maturity, all the plants in 1 m^2 were sampled at each plot, threshed and weighed after oven drying at 65 °C for 48 hours to determine biomass and seed yield per area with an analytical balance (Mettler, Toledo XP205DR, Greifensee, Switzerland). Seed number per square meter was counted using a *Contador* seed counter (Pfeuffer GmbH, Kitzengen, Germany), and average weight per sample was calculated as the average weight of three random subsets of 1000-seeds each. In Experiment NL 2013, seed yield per square meter, number of seeds per square meter and 1000-seed weight were calculated from the seed harvested from the whole container, and then extrapolated to yield per square meter. In Experiments CL 2014 and CL 2015, 1 m^2 was harvested in the central area of each plot. In all experiments, containers/plots were irrigated weekly to avoid water shortages, weeds were periodically removed by hand, while diseases and insects were prevented with fungicides or insecticides at the rates recommended by their manufacturers.

5.2.3. Association between seed yield and its components

To evaluate the association between seed yield and its main components under different N supplies, plant population densities and shading treatments at flowering, a regression analysis was performed. This was done by fitting a simple linear model to the relationship between yield (per silique, per plant or per unit area) with each one of its components. The slope of the linear model represents the rate of yield change and the intercept corresponds to the yield when the yield component is equal to zero. With this method, the association between i) silique yield and seeds per silique or single-seed weight was evaluated. The association between ii) seed yield per plant and the number of branches per plant, number of siliques per plant, number of seeds per plant or average seed weight per plant (1000-seed weight per plant), and between iii) seed yield per square meter and the number of seeds per square meter or 1000-seed weight were assessed.

5.2.4. *Plasticity of seed yield components*

Based on a framework designed for cereal crops (Sadras and Slafer, 2012), the plasticity of branch number, silique number, seeds per silique and average weight was evaluated and a ranking from the most plastic to the most conserved trait was obtained. Since one of the objectives of the current study is to quantify the sensitivity of the seed yield components of oilseed rape to variations in the resource availability, the relative changes in the number of stem branches per plant, in number of siliques per branch, in number of seeds per silique and in average seed weight were evaluated in response to i) increased plant population density (Experiment NL 2013 from 50 to 150 plants m^{-2}, Experiment CL 2014 from 40 to 80 m^{-2} and Experiment CL 2015 from 30 to 45 plants m^{-2}), reduced N supply (Experiment NL 2013 from 150 to 50 kg N ha^{-1}), and reduced incoming PAR at flowering (between beginning and end of flowering in Experiments CL 2014 and CL 2015). Since two levels of N supply, of plant population density and of incoming PAR were evaluated in the experiments, the plasticity of each of the yield components was calculated as the relative change (%) of the resource availability under the most restrictive condition (50 kg N ha^{-1}, 150 plants m^{-2} and shading by 70%) in relation to the highest resource availability (150 kg N ha^{-1}, 50 plants m^{-2} and full sunlight). This evaluation was done for each experiment separately.

5.2.5. *Response of yield components to source/sink ratio manipulations at flowering*

The research question on quantifying the response of seed yield to source/sink ratio manipulations at flowering was addressed by the FSP modelling approach. The model development, its adaptation to oilseed rape, and model use are described below.

5.2.6. *Model development and adaptation*

The effects of source/sink manipulations were addressed using an FSP model, developed for black mustard (*Brassica nigra*) (de Vries et al., 2018) and adapted for oilseed rape (see details in Chapter 3). Briefly, in this model the plant is represented in 3D as a compilation of phytomers where internodes, petioles, flowers and siliques are represented as cylinders, while leaf morphology is represented by a point cloud, converted into a 3D mesh surface. New organs are created as a function of temperature, and grow based on light capture, carbon assimilation and distribution of growing organs (leaves, internodes, flowers/siliques). Input parameters were collected from Experiment NL 2013, from the original model (de Vries et al., 2018) and from literature (Gabrielle et al., 1998; Labra et al., 2017). For further model details of source/sink approach, branch outgrowth and number of siliques, see Chapter 3.

5.2.7. Silique yield

Silique yield was calculated as a fraction of the total weight of each silique. After bud outgrowth, the flower and silique growth were determined by the C allocation from a common pool of assimilates; this flux is determined by the potential silique length. According to the experiments, 52 % of the silique weight corresponds to seed weight and 48 % to the external hull. Since these fractions were stable across the experiments and treatments, the current study assumed that 52 % of the silique weight was silique seed yield.

5.2.8. Source/sink manipulations at flowering

Two source/sink ratio manipulations simulated as: i) a reduction of source availability and ii) a reduction in sink strength, were compared with a control treatment under full sunlight. In the FSP model, a reduction in source availability was implemented between 53 and 72 days after plant emergence, which corresponds to the period between the beginning (BBCH 60) and end of flowering (BBCH 69). During this period, the total incident PAR including direct and diffuse radiation was reduced by 70%, mimicking the shading treatment performed in Experiments CL 2014 and CL 2015 (Chapter 4). The reduction of sink strength was implemented by removing 50% of the flowers after bud outgrowth. Flowers positioned at even phytomers were removed immediately after bud outgrowth, while flowers on the same branch but at odd phytomers were allowed to continue their growth.

5.2.9. Simulations

The growth and development of nine plants distributed in a grid of 3 × 3 plants (Fig. A5.1) were evaluated at a daily time step between emergence and physiological maturity. Each plot was cloned 20 × 20 times to minimize border effects. Treatments under contrasting N supply (N50 and N150) and plant density (D50 and D150) were simulated under full sunlight (Fig. A5.1a), source limitation (i.e. shading) (Fig. A5.1b) and sink strength limitation (i.e. flower removal) (Fig. A5.1c). The effects of these manipulations on plant source/sink ratio and plant biomass were evaluated. The next step was the quantification of their effects on the yield components: number of branches per plant, number of siliques per plant and number of siliques in the main raceme. Finally, the impacts on seed yield per plant and per unit area were evaluated.

5.3. Results

5.3.1. Seed yield and its components recorded in the experiments

The association between seed yield and its main components was evaluated per silique, per plant and per unit area. To evaluate the relationship between silique yield and number of seeds per silique, two independent regressions were fitted (Fig. 5.1a): one for treatments under full sunlight ($r=0.93$) and another for treatments under shading ($r=0.97$). Treatments under shading showed a steeper slope ($P<0.05$) than treatments under full sunlight. It was observed that the higher the seed number the higher the single-seed weight increase, especially in treatments under shading. The single-seed weight under shading, calculated across all treatments, was 67% heavier than seeds in treatments under full sunlight (Fig. 5.1b). In general, the highest yields and number of seeds per silique were observed in the “oldest siliques” positioned at the bottom of the branches, while the lowest yields were in the “youngest” siliques positioned at the top of the branches.

Yield per plant was closely associated with the number of branches per plant (Fig. 5.1c), with the number of siliques per plant (Fig. 5.1d) and with the number of seeds per plant (Fig. 5.1e), but in the last, two regressions were fitted to treatments either in control (full sunlight) and under shading treatments. The degree of association of yield with these three traits is remarkable, considering the type of treatments included and the broad range of environmental conditions under which the experiments were performed (i.e. under full sunlight vs shading, different N supplies and plant densities, in the Netherlands and in Chile, under open-sided shelter vs field). In contrast, the association between yield per plant and 1000-seed weight was less straightforward (Fig. 5.1f). For instance, when the treatments were not exposed to source limitation at flowering, the 1000-seed weight, which on average was 3.2 g, varied in a slightly narrower range between -13 and 6%, but when shading was performed at flowering, seed weight was increased by a range between 37 and 65%.

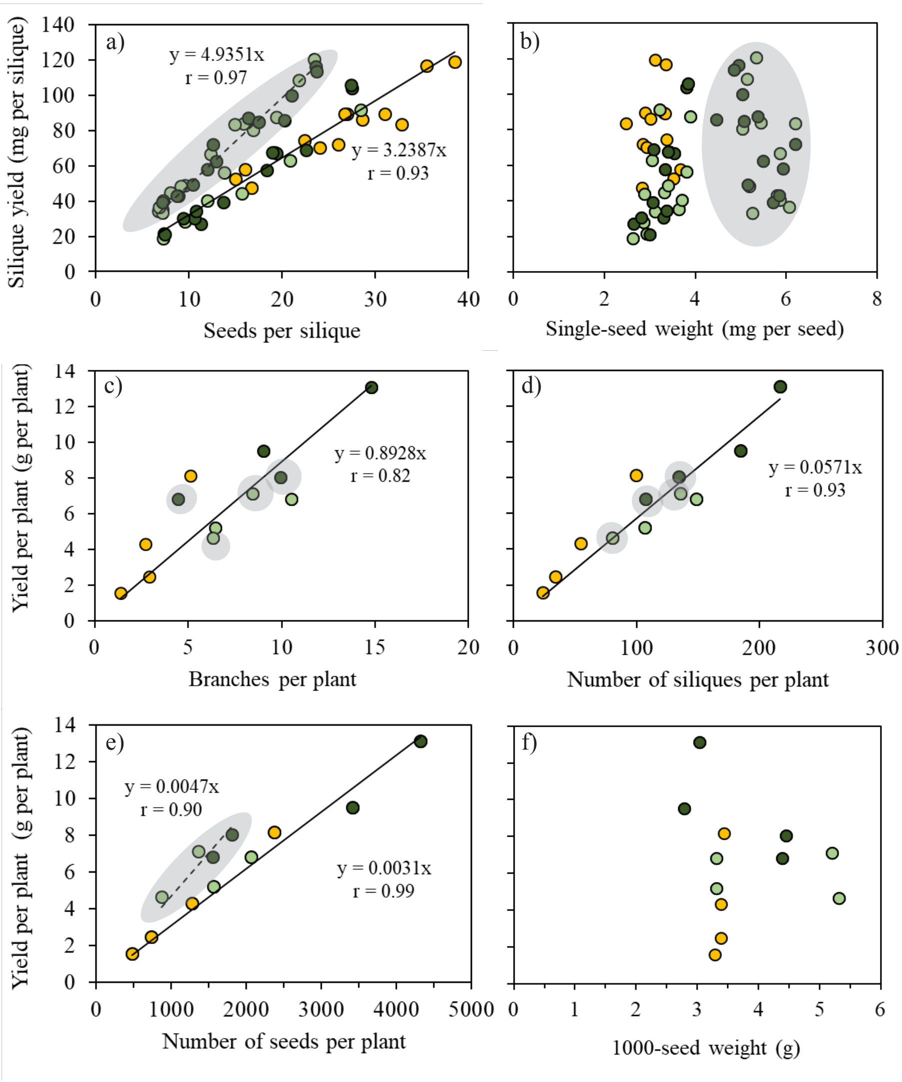

Fig. 5.1. Relationship between: a) seed yield per silique and seed number per silique, b) seed yield per silique and single-seed weight, c) seed yield per plant and number of branches per plant, d) seed yield per plant and number of siliques per plant, e) yield per plant and number of seeds per plant, f) seed yield per plant and 1000-seed weight, in response to N supply, plant density and shading at flowering. NL 2013, CL 2014 and CL 2015 represent the experiments performed in the Netherlands in the year 2013, Chile 2014 and Chile 2015 respectively. Gray circles identify the treatments exposed to shading at flowering.

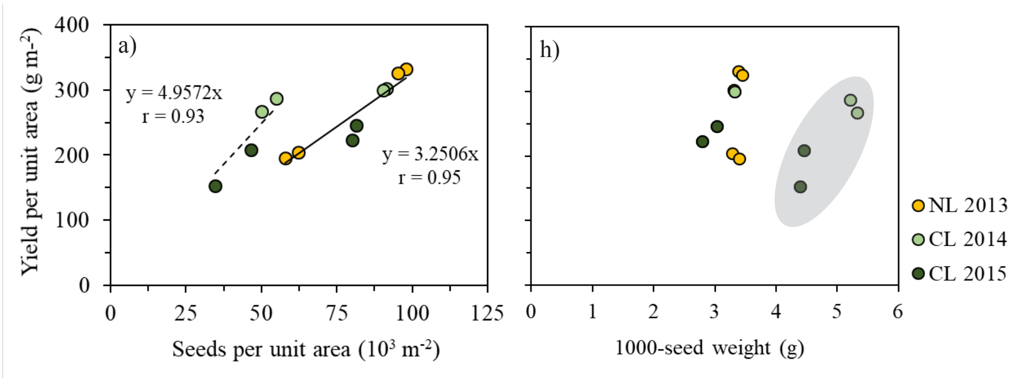

Fig 5.2. Relationship between a) seed yield per unit area and number of seeds per unit area, b) seed yield per unit of area and 1000-seed weight, in response to N supply, plant density and shading at flowering. NL 2013, CL 2014 and CL 2015 represent the experiments performed in the Netherlands in the year 2013, Chile 2014 and Chile 2015 respectively. Gray circles identify the treatments exposed to shading at flowering.

Yield per unit area was better associated with the number of seeds per unit area (Fig. 5.2a) than with 1000-seed weight (Fig. 5.2b). As performed in the analysis per silique and per plant, to evaluate the relationship between yield per unit area and number of seeds per area, two regressions were fitted accounting for the data recorded in control (full sunlight) and under shading treatments. A steeper slope ($P<0.05$) was observed in treatments under shading than at full sunlight. Additionally, higher seed yield ($P<0.05$) was observed in high N supply treatments than at low N, while plant density showed ambiguous effect ($P>0.05$) on yield per unit area.

5.3.2. Plasticity of seed yield components

The relative changes in number of branches per plant, number of siliques per plant, number of seeds per silique and seed weight were quantified for each trait in response to plant density, N supply and shading at flowering (Fig. 5.3). Plant densities of 50 and 150 m^{-2} (Experiment NL 2013), 40 and 80 m^{-2} (Experiment CL 2014) and 30 and 45 plants m^{-2} (Experiment CL 2015) caused reductions in the number of siliques per plant in a range between 13-61 %, while number of branches was reduced by 32-46 %, and number of seeds per silique by 13-22 %. Since in Experiment CL 2015, plant population was not contrasting enough, plant phenotype remained unaffected by intra-specific competition, with a variation close to zero for all traits. So, Experiment CL 2015 was not considered as part of these ranges of variations. A reduction of N supply from 150 to only 50 kg N ha^{-1} reduced the number of branches by 49 %, siliques per plant by 38 % and seeds per silique by 6 %. Shading at flowering reduced the number of branches by 11-30 %, siliques per plant by 17-39 % and seeds per silique by 6-28%. Thousand seed weight varied only in a range between 0.5 and 10 % in response to N supply and plant density, but it was increased from 64 to 70 % in response to shading at flowering. Therefore, the ranking of plasticity of seed yield components of oilseed rape was as follows: average seed weight > number of siliques per plant > number of branches per plant > number of seeds per silique, and this hierarchy is mainly influenced by the source limitation at flowering.

5.3.3. Effects of simulated source/sink ratio manipulations at flowering on yield components

The time-course of the simulated source/sink ratio during the crop cycle showed a slow but consistent increase across treatments between plant emergence and budding stage, followed by a sharp decrease (Fig. 5.4a and b). This marks the end of the vegetative stage and the beginning of the reproductive stage, i.e. the moment when new and strong sinks start their outgrowth and further development. At this stage, axillary buds break their dormancy in response to factors such as intercepted PAR, red: far red ratio and sugar signalling (Leduc et al., 2014), modulated by hormone balance (Domagalska and Leyser, 2011), which trigger the development of branches and flowers, causing an abrupt decrease in the source/sink ratio (Fig. 5.4a, b). Another increase in this ratio takes place after budding stage; the ratio reaches its maximum value between middle and end of flowering, followed by a decrease due to leaf senescence and leaf litter until values close to zero. The source-limitation (Fig. 5.4a) simulated by a canopy shading between 721 and 1045 °C d caused a reduction in the source/sink ratio, and its final decline was observed earlier than in the control for all combinations of N supplies and plant densities. The reduction of sink strength (Fig. 5.4b), simulated by the removal of 50% of the flowers at each main raceme and branch caused a small increase in the source/sink ratio, delaying the final decline, due to the reduction in the number of siliques set in the upper canopy allowing a higher light penetration inside the canopy, increasing the light intercepted by the remaining leaves, the most important C sources.

One of the effects of shading at flowering was the reduction of plant biomass at maturity (Fig. 5.4c), in a range between 11 and 21% for N50-D50 and N150-D150, respectively, in comparison with the control. In contrast, simulated flower removal (Fig. 5.4d) caused an increase in biomass in a range between 1.4 and 8% in N150-D50 and N150-D150, respectively.

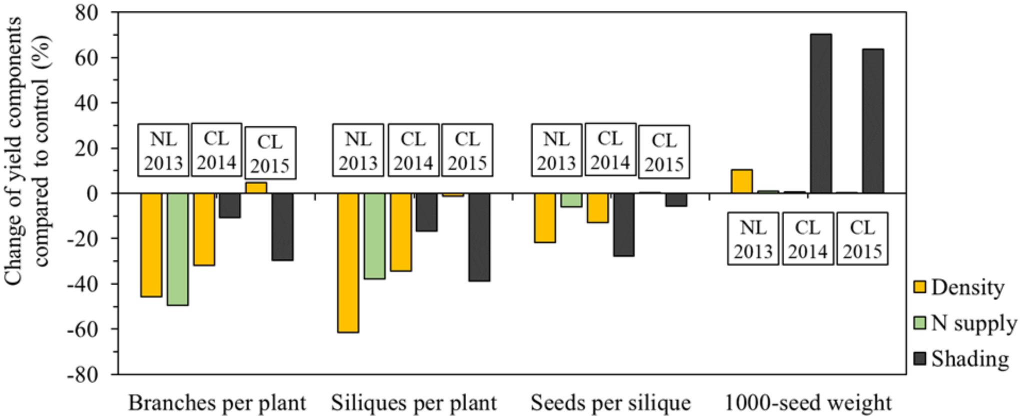

Fig. 5.3. Relative change of stem branches per plant, number of siliques per branch, seed per silique and 1000-seed weight, in response to increased plant population density, shading and N supply in Experiments NL 2013, CL 2014 and CL 2015.

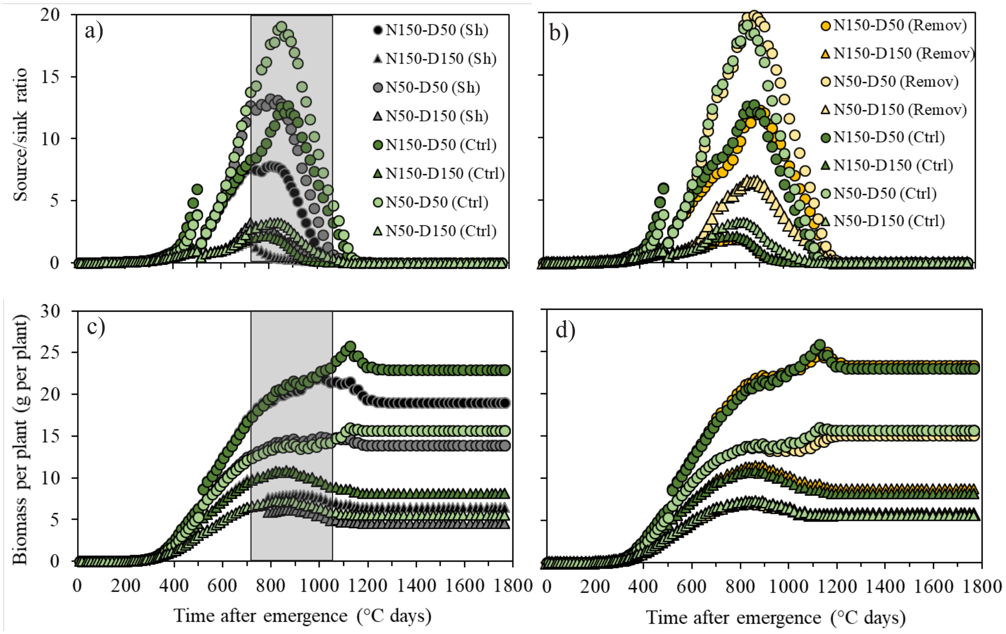

Fig. 5.4. Simulated plant source/sink ratio of treatments under full sunlight (Ctrl) vs a) under treatment under shading (Sh) and b) flower removal (Remov), and simulated plant biomass of treatments under full sunlight vs c) under shading, d) flower removal, between plant emergence and physiological maturity and under contrasting N supplies and plant densities. Gray area in panels identify the period during which treatments were shaded.

The FSP model allowed to simulate properly the number of branches per plant in response to N supply and plant density as demonstrated in Chapter 3, while the virtual source/sink ratio manipulations at flowering did not affect the stem branching pattern (Fig. 5.5a). The highest number of branches per plant was observed in treatment N150-D50, followed by N50-D50 and N150-D150, while N50-D150, the treatment grown under the most restrictive conditions, recorded the lowest number of branches. Another important effect of virtual shading at flowering was the reduction in the number of fertile siliques per plant (Fig. 5.5b). This was especially noticeable at high plant density, where the number of siliques per plant was reduced by 37 and 59 % for N150-D150 and N50-D150, respectively, in comparison with the unshaded control, while it was reduced by 12 and 26 % for N150-D50 and N50-D50. Flower removal reduced the number of siliques per plant by 50 % in all cases.

An analysis of siliques positioned along the main raceme (Fig. 5.6) showed that the highest silique yields were reached by siliques positioned at the bottom of the branch, corresponding to the oldest siliques. The siliques with the highest yield were found in treatment N150-D50 (Fig. 5.6a), followed by N50-D50 (Fig. 5.6c), and N150-D150 (Fig. 5.6b), while the siliques with the lowest yield were observed in N50-D150 (Fig. 5.6d).

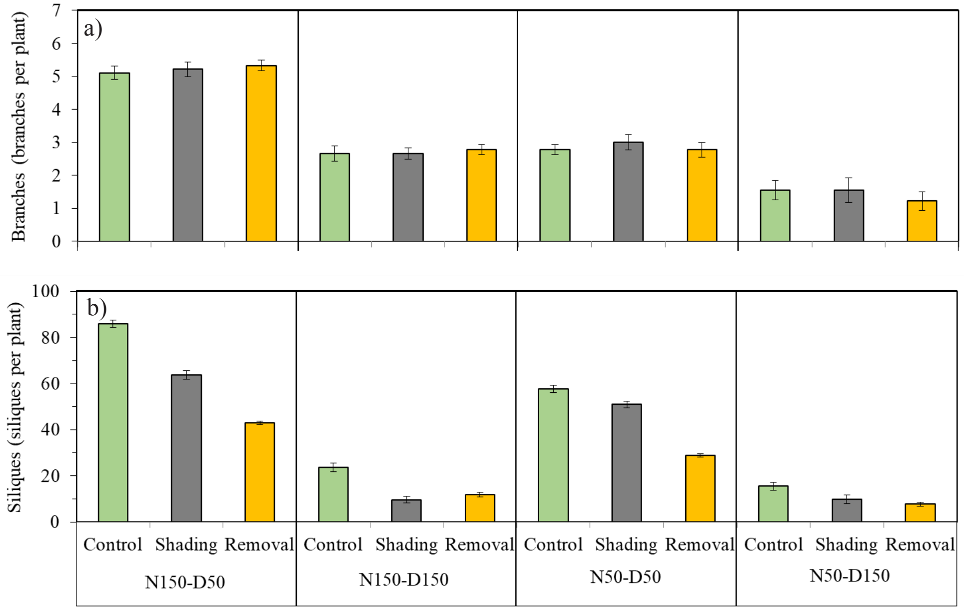

Fig. 5.5. Simulated a) number of branches per plant, b) number of siliques per plant, for control, shading and flower removal treatments, under contrasting N supplies and plant densities.

Shading at flowering caused a reduction in silique yield from 32-40 % at the top of the branch, until 50-63 % in siliques positioned at the bottom of the branch, averaged across all combinations of N supply and plant density.

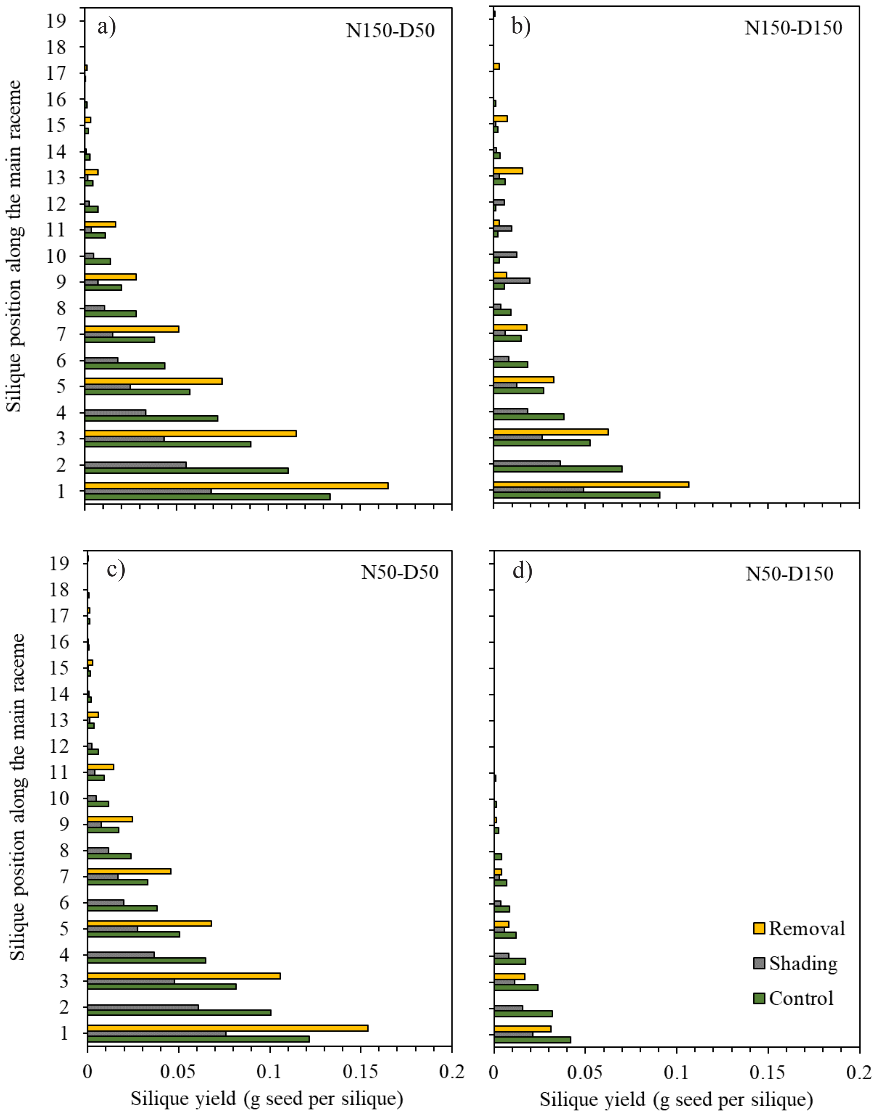

Fig. 5.6. Yield of siliques positioned along the main raceme for a) N150-D50, b) N150-D150, c) N50-D50 and d) N50-D150, in response to flower removal, virtual shading and control under full sunlight.

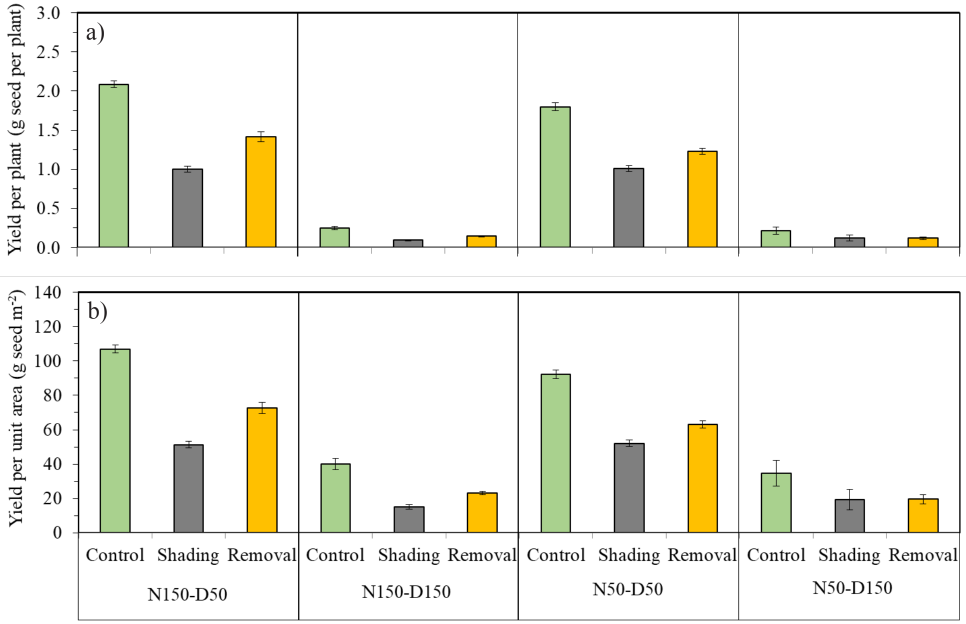

Fig. 5.7. a) Plant yield and b) yield per unit area, for treatments control, virtual shading and flower removal, and under contrasting N supply and plant density.

Flower removal reduced the number of siliques by 50 %, but in treatments N150-D50 and N50-D50, the yield of siliques positioned at the bottom was 24 and 27 % higher than in the control, and in siliques positioned at the top of the branch the silique yield was 70 % higher than in the control, partially compensating the reduction in silique number. In the specific case of treatment N50-D150, flower removal did not increase yield in siliques set at the top of the branch. Shading affected more the yield of plants grown at high N than the yield at low N (Fig. 5.7a). Indeed, the reduction of yield per plant in response to shading was 52 and 62 % in N150-D50 and N50-D50, while for both N50-D50 and N50-D150 it was 44 %. On the other hand, the treatment most affected by flower removal was N150-D150 with a reduction of 42 % in plant yield, while reductions of 32 % were observed in N150-D50 and N50-D50, and of 19 % in the case of N50-D150. The magnitude of these differences between individual plants were also observed at crop level (Fig. 5.7b).

5.4. Discussion

5.4.1. Seed yield and its components

The first aim of this study was to identify the seed yield components most associated with seed yield in oilseed rape. Variation in yield per unit area was to a high extent accounted for by variation in the number of seeds per unit area, while there was not a clear relationship between yield per unit area and 1000-seed weight. The same behaviour has been observed in crops such as wheat, maize, sunflower and soybean (Sadras, 2007; Sadras and Denison, 2009), where the plant reproductive output largely relies on the adjustment by seed number.

To understand the origin of these relationships, analyses of plant yield and silique yield were performed in oilseed rape. Plant yield was to a great extent explained by three yield components: number of siliques per plant, number of seeds per plant and number of branches per plant, while silique yield was explained by number of seeds per silique, confirming earlier results (Lu et al., 2011). The close association between seed yield and yield components mentioned above was noticeable, considering the treatments imposed and the contrasting environmental conditions under which these experiments were carried out (i.e. under full sunlight vs shading, different N supplies and plant densities, under open-sided shelter vs field, in the Netherlands and Chile).

In contrast, the association between seed yield and seed weight was less clear. Average seed weight did not vary among treatments managed with contrasting N supplies or plant densities, nor did it vary when experiments were performed in different environments. But this does not mean seed weight is a conserved trait, taking into account that under source limitation at flowering seed weight was increased up to 2-folds compared with the treatments under full sunlight Chapter 4, leading to an increase in the average seed weight by 60% and compensating for reductions in number of seeds per unit area.

Interestingly, the steeper slope in the relationship between seed yield and seed number and the heavier seeds observed consistently (i.e. per silique, per plant and per unit area) in treatments under shading versus treatments under full sunlight, revealed that the higher the seed number the higher the seed weight increase. This suggests that seed weight is not 100% source-limited at flowering, but a degree of co-limitation by source and sink can play an important role at this stage.

5.4.2. Plasticity of seed yield components

Plant performance depends on the plasticity from basic to complex traits. Basic traits correspond to response mechanisms/sensitivities (e.g. photosynthesis, sensitivity to photoperiod), while complex traits integrate a larger number of processes over time (e.g. biomass, partitioning of biomass, seed yield components), varying in contribution to adaptation across environmental conditions according to their plasticity (Bustos-Korts et al., 2019; Slafer et al., 2005). The results obtained in our study shows the following ranking of plasticities for oilseed rape: 1000-seed weight > number of siliques per plant > number of branches per plant > number of seeds per silique, which is different from the ranking proposed by Sadras and Slafer (2012) for cereals. In oilseed rape, 1000-seed weight is at the top of the hierarchy exclusively due to the 60 and 70% of heavier seeds observed in treatments shaded at flowering, since this trait did not respond to any other environmental factor manipulated in the study (i.e. plant density, N supply, weather conditions in the different experiments). This level of plasticity in seed weight has never been reported for annual grain crops, since most of the studies considered it as the most conserved plant trait (Peltonen-Sainio et al., 2007b; Sadras, 2007; Sadras and Denison, 2009; Turnbull et al., 1999) and this was assumed to be true for other crops like oilseed rape. The number of siliques per plants is known to be one of the most plastic traits in oilseed rape (Lu et al., 2011), as well as the number of branches per plant, but the last one was only at the third place in the ranking, while the number of seeds per silique was the most conserved trait.

It was demonstrated that the degree of trait plasticity in oilseed rape is highly dependent on the type and timing of source limitations, but also depends on several other factors such as crop, genotype, environment and genotype × environment interaction. For instance, in long-term experiments evaluating cultivars of wheat, rye, oat, barley and oilseed rape released in the last 40 years, it was shown that seed yield and seed number of winter rye are more plastic than those in wheat, oat or oilseed rape, while these traits are more stable in six-row barley. Time-trends in plasticity also revealed that the association between yield plasticity and average seed weight tended to change over the years in two-row barley (Peltonen-Sainio et al., 2011). Traits underlying yield also differ in their contribution to adaptation across environmental conditions and have different genotype × environment interaction. Plastic responses of yield and physiological traits associated across several environments in Australia were generated and analysed in 199 genotypes of wheat using the crop growth model APSIM-wheat (Bustos-Korts et al., 2019), concluding that the order of yield component relevance for yield changes across environments. Therefore, the ranking of plastic responses among yield components is not static, but can vary in response to crop, genotype, environmental conditions and their interactions, but also to the nature and timing of the source limitations as found in oilseed rape.

5.4.3. Effects of simulated source/sink ratio manipulations at flowering

Since it has been hypothesized that the number of siliques per plant and the yield per silique, two of the traits most related with oilseed rape yield, are mainly determined by plant C status at flowering stage (Kirkegaard et al., 2018; Wang et al., 2016), this study evaluated the plasticity of number of siliques per plant, yield per silique, plant or crop in response to simulated source/sink ratio manipulations at flowering, using an FSP modelling approach. Source/sink manipulations were simulated by reducing source availability at flowering (shading), as performed in previous experiments (Labra et al., 2017; Verdejo and Calderini, 2020) and by reducing sink strength (flower removal) without affecting plant architecture (i.e. branching).

The model allowed to simulate different plant C status for oilseed rape at flowering, under contrasting scenarios of N supply and plant density, without affecting stem branching pattern. In high-yielding environments, seed yield is primarily determined by biomass (Zhang and Flottmann, 2016), and in the current study, the source limitation at flowering reduced plant biomass, especially in plants grown at low intraspecific competition. It has been observed that crop biomass status at vegetative, budding, flowering, pod setting, and physiological maturity as well as the crop growth rate between budding and silique setting are good predictors of the number of siliques per unit area and number of seeds per unit area (Zhang and Flottmann, 2016). In contrast, flower removal improved the light distribution through the canopy, slightly increasing the light intercepted by green leaves performing photosynthesis (Fig. A5.2b), with a minor increase in plant biomass. From a simulation study using LINTUL-BRASNAP (Habekotté, 1997b), it was suggested that optimizing light distribution inside the canopy during the reproductive stage by i) reducing the flower petals size and ii) having more erect clustered siliques could be a breeding strategy to increase seed yield. However, according to our study biomass gain due to improved light penetration inside the canopy is only marginal, while the benefit on yield was null.

The number of siliques per plant was heavily affected by shading, especially at high plant density, where shading reduced the number of siliques per plant by 37-59 % in comparison with the control under full sunlight, while flower removal reduced the number of siliques per plant by 50 %, which was indeed the original purpose of this treatment. Since the highest number of flowers, siliques and seeds per silique and per plant are produced on the main raceme (Tayo and Morgan, 1975; Wang et al., 2011), making it the highest contributor branch to plant yield, the analysis of silique yield focused on the main raceme in this study. The yield of siliques positioned at the bottom of the main raceme was 20 % more affected by shading than the yield of the siliques positioned at the top of the main raceme. Since the oldest siliques are set at the bottom, and their development and growth happened under source limitation, it is logical that they were more affected than the youngest siliques positioned at the top, which were only partially affected by, or not exposed to, shading at all. Although flower removal reduced the number of siliques by 50 %, this was partially compensated by the increase in yield per silique, but in different magnitudes depending on the position along the branch, N supply and plant density. This partial compensation was mainly observed in siliques positioned at the top of the branch (70% more yield than in the control), in comparison with siliques at the bottom (ca. 25 % more yield than in the control) and it was observed in most of the combinations between N supply and plant density, but not under the most restrictive conditions (N50-D150).

The results obtained by the current modelling approach suggest that the amount of assimilates available at flowering is the primary determinant of plant yield, while silique yield is explained by the position of the silique on the branch and the time of both branch and silique appearances. This is supported by an experiment (Wang et al., 2011) where the number of siliques, the number of ovules per silique and the number of seeds per silique were mostly explained by the plant C availability at flowering. Process-based models like APSIM-CANOLA take this into consideration by simulating seed yield per unit area based on the crop biomass status at flowering with accurated results (Farre et al., 2002; Robertson and Lilley, 2016). But in this type of models the prediction of seed yield components is mainly based on empirical coefficients. For instance, in APSIM a fraction of biomass at flowering determines the number of seeds per unit area at maturity, while 1000-seed weight depends on the variations in the crop biomass during seed filling. Other crop growth models, such as LINTUL-BRASNAP (Habekotté, 1997a), CERES-RAPE (Gabrielle et al., 1998) and CECOL (Husson et al., 1998), also simulate crop growth and seed yield of oilseed rape in response to the environment or agronomic management, but all of them estimate C assimilates as product of absorbed PAR and radiation use efficiency (RUE). In addition, none of them considers plant/crop architecture or its interaction with physiological processes, which is of paramount importance to explore the plasticity of yield components in response to source/sink manipulations in plastic crops like oilseed rape. Since the FSP models explicitly describe the development over time of the 3D architecture of individual organs, as governed by physiological processes, which depend on environmental factors (Evers and Marcelis, 2019; Vos et al., 2010), these models allow to analyse dynamic feedbacks among organs, to finally upscale these processes to plant and plant population, being a helpful tool to quantify the plant response to the environmental factors. For instance, the effects of variations in plant source/sink status caused by foliar diseases, herbivory, global dimming, agronomic practices, or breeding manipulations on seed yield can be simulated integrating processes from silique-to-crop.

5.4.4. Further work

The need to understand the plasticity in, and interactions among, yield components "is obvious because they impose constraints on the form of a plant, and are frequently barriers to improve seed yield" (Grafius, 1978), for which an analytical approach based on plant/crop modelling is crucial. One of the main challenges for modellers is to design the key algorithms for an accurately estimation of the source-sink interactions, in order to simulate seed number and weight (Asseng et al., 2017). However, this will be possible only after elucidating the physiological basis that trigger the expression of plasticity of seed yield components. For instance, further research should investigate whether the expression of heavier seeds, in response to the reduction of assimilates at flowering, is triggered by a switch in the potential seed weight, the influx of more assimilates towards the seeds for a sink reduction through the lower seed number when the incoming PAR is again increased to 100% or due to the role of non-resource signals (Chapter 4). In our modelling approach several interactions among plant organs aboveground were considered, but roots were excluded. Since it is known that practices like plant density modify plant root architecture, and in turn, nutrient uptake (Hecht et al., 2016), future model evaluations should also include interactions belowground (Evers et al., 2019).

Improving the understanding of the source/sink dynamics, together with more accurate modelling, is important for estimating crop-environment interactions that affect assimilate supply (Asseng et al., 2017). Then, optimization of agronomic practices and future breeding strategies can be directed to increase assimilates availability during stages when source limitation is critical like for example flowering stage (Zhang and Flottmann, 2018).

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Supplementary Material

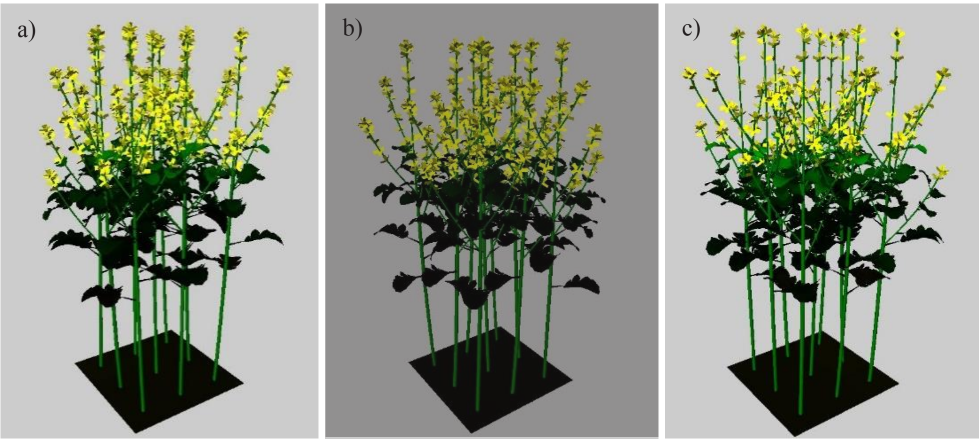

Fig. A5.1. Example of visual output of treatment N150-D50 a) under full sunlight, b) under shading and c) flower removal.

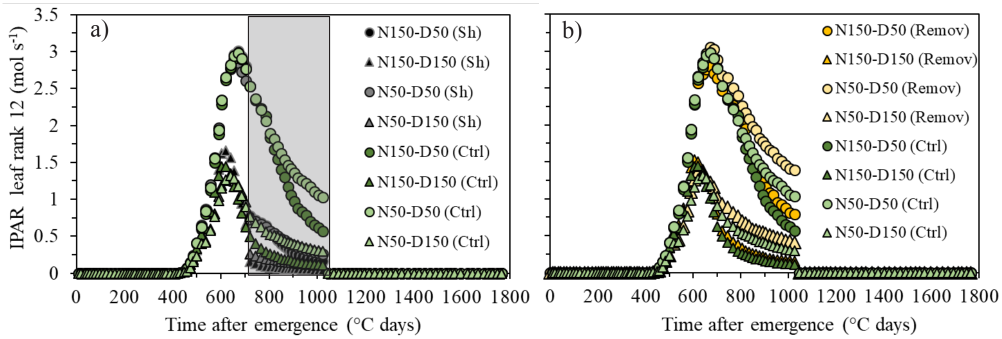

Fig. A5.2. Simulated PAR intercepted by leaf ranking 12 under full sunlight vs a) shading (Sh) and b) flower removal (Remov), between plant emergence and physiological maturity and under contrasting N supplies and plant densities. Gray area in panels identify the period during which treatments were shaded.

Chapter 6

General discussion

6.1. Phenotypic plasticity in oilseed rape in response to N supply, plant density and source-sink ratio manipulations

The objective of this thesis was to evaluate the plasticity in the responses of oilseed rape to environmental factors and agronomic practices. This evaluation was carried out by identifying the main drivers and mechanisms underlying plant C and N distribution, and analysing the consequences of C and N distribution on plant architectural traits and potential interactions with traits associated with seed yield. I implemented combined experimental and modelling approaches to answer the following research questions:

Q1. To what extent does the interaction between N supply and plant population density alter N distribution and leaf traits in oilseed rape?
Q2: What is the importance of N fertilizer supply in branch and siliques outgrowth?
Q3: Can seed weight compensate for reductions in seed number in response to artificially induced source limitation at flowering?
Q4: Do the seed yield components of oilseed rape differ in their plastic response to N supply, plant density and plant C status at flowering?

In this chapter, the relevance of the results obtained in this thesis are discussed, the potential applications in breeding and crop management are proposed and the approach used in this thesis is analysed.

6.1.1. Contribution of this study to improving knowledge of plasticity in oilseed rape

Since environments are highly variable in space and time (Valladares et al., 2007), plants must acclimate to variations in light intensity, air temperature and water vapor deficit (Pedruzo-Bagazgoitia et al., 2017; Sikma et al., 2018), but also to variations in soil physical characteristics, and nutrients and water availability during their life cycle (Tuffour et al., 2016; Vasu et al., 2017). This stimulates plant plastic responses to adapt the plant phenotype to the local conditions. Based on their plastic response to the environment, organisms can be defined as generalists or specialists (Van Tienderen, 1991), assuming that advantages of being a specialist in one environment are accompanied by disadvantages in another, and considering phenotypic plasticity as one of the components of being a generalist. Several studies have evaluated plastic responses of generalists (Agusti and Greb, 2013; Murren et al., 2015; Pfennig et al., 2010; Richards et al., 2006; Wund, 2012), while specialists have received less attention. A recent study (Bongers et al., 2019) suggested that single plastic responses may not evolve, but there is variation in plastic responses depending on the environment the species are selected in, which goes against the notion that phenotypic plasticity is mostly a characteristic of generalists.

Because oilseed rape is bred to express a superior agronomic performance in a specific environment (Thurling, 1991), it can be classified as a specialist. However, several studies have demonstrated a high plasticity in oilseed rape in response to environmental factors (Franzaring et al., 2008; Muller et al., 2012; Takashima et al., 2013) and agronomic practices (French et al., 2016; Khan et al., 2018; Ren et al., 2017). The current study explored the response of oilseed rape plants to environmental factors like light, and agronomic practices such as N supply and plant population density during key phenological stages. For this purpose, N distribution, C assimilation and leaf morphology were evaluated during the vegetative stage (Chapter 2), and during stem branching at budding stage (Chapter 3), while the interaction between plant C status and seed yield components such as number of siliques per plant, number of seeds per silique, and single seed weight were also considered in this analysis (Chapter 4 and Chapter 5).

Two of the agronomic practices with the highest impact on oilseed rape production are N supply and plant population density (Kuai et al., 2016), which affect plant N and C distributions during the plant life cycle. In plant canopies, leaf N distribution follows the light distribution, in an exponential decline over the canopy depth (Fig. 1.1a), explained by the Beer`s law (Hirose, 2005; Monsi and Saeki, 2005), which agrees with the optimal distribution of leaf N to maximize C assimilation and crop productivity (Dreccer et al., 2000). The relationship between leaf N content and photosynthetic rate per unit leaf surface has been investigated in a range of different plant species (Bertheloot et al., 2008; Dreccer et al., 2000; Field, 1983; Hikosaka, 2016; Hirose, 2005; Li et al., 2013; Monsi and Saeki, 2005), but so far not in crops like oilseed rape (Gammelvind et al., 1996). Thus, Chapter 2 evaluated the importance of N fertilizer supply on plant N distribution and whether N supply changes plant investment in leaf area or leaf mass per area in oilseed rape. To achieve this objective, spring oilseed rape was grown at two rates of N supply (50 and 150 kg ha^{-1}) and two plant population densities (50 and 150 plants m^{-2}) in containers, under an open-sided shelter. Traits such as plant height, photosynthesis, leaf area, biomass and N content of selected leaves were measured at budding stage. N supply determined

N content per unit of leaf mass (N_{mass}) and leaf area, with important effects on light distribution and C assimilation at crop level. But N supply played a subordinate role in altering N per unit of area (N_{area}), the main determinant of photosynthesis per unit of leaf area (A_{n}). Leaf mass per unit of leaf area (*LMA*) was affected by an interaction between N supply and plant population density. This interaction between N and light can have effects on leaf area index, one of the determinants of the amount of photosynthetic active radiation (PAR) intercepted by the plant canopy (Kuai et al., 2016), with potential effects on light distribution and C assimilation at crop level.

Another important factor affecting light distribution is the stem branching pattern, but branching is itself determined by light. This is the case as the number of branches per plant depends on the number of axillary buds along the stem that break their dormancy (Wang and Li, 2008). It is known that bud outgrowth is influenced by several internal and external factors, among which PAR, plant C status (Leduc et al., 2014), red: far red ratio (Ballaré et al., 1990; Rondanini et al., 2014), sugar content and their interaction with the concentrations of hormones such as auxins, strigolactones and cytokinins (Domagalska and Leyser, 2011; Rameau et al., 2015), have been identified as the main ones responsible for bud outgrowth and subsequently for stem branching. It is also known that N is associated with C assimilation (Hikosaka et al., 2016) and meristematic activities (de Jong et al., 2014), but there is no conclusive evidence of a direct role of N availability on bud outgrowth (Rameau et al., 2015). Since the number and spatial distribution of stem branches determine plant architecture and plant source/sink ratio, (Jullien et al., 2011; Pinet et al., 2015; Rondanini et al., 2017) and the number of seeds per silique (Wang et al., 2011), the agronomic performance of oilseed rape could be determined by the interaction between stem branching and plant source/sink ratio. This hypothesis was addressed in Chapter 3, where the effect of N supply as determinant of plant source-sink status, on branch outgrowth and silique production was evaluated by combining experimental and Functional-Structural Plant (FSP) modelling approaches. An FSP model was adapted for oilseed rape and branch and silique outgrowth were implemented as dependent on the plant source/sink ratio status, mainly driven by light interception. The plant source/sink ratio at budding stage was more responsive to variations in plant density than to variations in N supply, being in my study 50% higher at low population density than at high plant density. This suggests that plants at high intraspecific competition trigger branch outgrowth at lower source/sink ratio than at lower intraspecific competition. The number of branches per plant and the number of siliques per plant simulated by the FSP modelling approach in Chapter 3 were similar to the measured numbers. Therefore, the hypothesized mechanism that the number of siliques per plant is determined by the source/sink ratio, which in itself is affected by the number of branches per plant, was confirmed. Indeed, the plant C status at budding stage, reflected by the plant source-sink ratio, was an accurate indicator of the number of branches per plant at physiological maturity, and both define the number of siliques per plant. The effect of the number of siliques on source-sink ratio has two separate stages: i) initially, green siliques are a net source of C assimilates making an important contribution to total assimilate production because of their photosynthetic activity, but after reaching its maximal size, ii) then become net sinks, competing for assimilates with other sinks like for example branches.

Additionally, the hypothetical limits of oilseed rape plants to produce siliques under a simulated branch inhibition were explored in Chapter 3. The objective of this exercise was to evaluate the importance of stem branching in plant productivity, considering number of siliques per plant as a proxy of plant yield. For this purpose, the number of siliques produced by plants with only one branch (the main raceme) was compared with the number of siliques of a plant with multiple branches. The results showed that the main raceme of an oilseed rape plant has the potential to develop a similar number of siliques as a plant with several branches. However, to achieve this full compensation, the model assumed several phenomena that are not very realistic. For example, the plant life cycle should be extended by a factor four (this means until the winter season), with no limitations of water or nutrients during that period of time, no negative effects of short photoperiods or low temperatures prevalent in winter, and without any plant mechanical or physiological constraints (i.e. no limits to height of plants, no risk of lodging, no problems associated with differential maturity of siliques at the bottom and at the top of the main raceme). This suggests that stem branching pattern is important for maximizing silique production by for example allowing the plant to produce a higher number of siliques in a shorter time than the plants with inhibited branching.

Chapter 3 assumed branch development and silique production as the only compensatory mechanisms in response to variations in C availability, but oilseed rape can adapt its phenotype in response to resource availability and environmental signals from early developmental stages by, for example, modifying the diameter of the rosette, leaf size, petiole length (Rondanini et al., 2017; Rondanini et al., 2014), or, even later in the plant growth cycle, by altering the stem branching pattern (Pinet et al., 2015; Wang et al., 2011) or, even at flowering, by adjusting the number of siliques per plant, and the number of seeds per silique (Kirkegaard et al., 2018; Wang et al., 2011). It is assumed that resource limitation has a negative effect on plant productivity (Valladares et al., 2007), but the responses of the seed yield components, such as the number of seeds per unit area, single and average seed weight, and quality traits, such as oil content, to source limitation at flowering have been scarcely assessed in oilseed rape (Tayo and Morgan, 1979). Chapter 4 addressed the question: Can seed weight compensate for reductions in seed number in response to artificially induced source limitation at flowering? It was found that single and average seed weight did not vary among treatments managed with contrasting N supplies or plant densities, nor did it vary when experiments were performed in different environments. But under source limitation at flowering, single seed weight was increased up to twofold compared with the treatments under full sunlight, leading to an increase in the average seed weight by 60% and compensating for reductions in number of seeds per unit area, without affecting oil or protein concentrations. The magnitude of plasticity in seed weight found in this chapter on oilseed rape has never been reported before in literature for this or any other grain crop (Kiniry, 1988; Sadras, 2007; Tayo and Morgan, 1979), opening new questions such as:

What are the mechanism underlying the increase in potential seed weight in oilseed rape particularly at flowering?
Is the increase in potential seed weight triggered by resources or signals?
Is this behaviour crop or genotype dependent?
What factors determine that single seed weight achieve a partial (Kirkegaard et al., 2018; Verdejo and Calderini, 2020; Zhang and Flottmann, 2018) or full (Chapter 4) compensation to reductions in seed number per silique?

This study also hints at the necessity of re-evaluating the paradigm assuming that seed number per unit area is the main source of variation of seed yield, while seed weight is mostly a conserved trait (Sadras, 2007), and for modelling purposes can be considered constant. Indeed, how stable or plastic a single plant trait is, depends on the availability of resources, and on environmental signals, but also on the moment at which resources are limited.

Chapter 5 built on Chapter 4 and integrated elements addressed in Chapters 2 and 3 by
a) identifying the seed yield components that account for variation in seed yield and quantifying

their sensitivity to resource availability, using datasets collected from three experiments; and b) evaluating the plastic response of seed yield to source/sink ratio manipulations at flowering using an FSP modelling approach.

Variations in seed yield per unit area were mostly explained by the number of seeds per unit area, while seed yield per plant strongly associated with variations in the number of branches per plant, number of siliques per plant and number of seeds per plant, while yield per silique was explained by the number of seeds per silique. But this does not mean single seed weight is a conserved trait, taking into account that under source limitation at flowering single seed weight was increased up to twofold compared with the treatments under full sunlight. Indeed, when comparing the stability of the yield components of oilseed rape in response to source limitation at flowering, single seed weight was classified as the most plastic trait. However, this was the most stable trait in response to all the other environmental factors evaluated in this thesis (i.e. plant density, N supply, weather conditions in the different experiments). This suggests that the sensitivity of plant traits to environmental factors is not static and dependent on the crop, the type and timing of source limitation. In the same chapter, different plant source/sink ratios were simulated by an FSP model previously adapted in Chapter 3 for oilseed rape. The simulated shading caused a reduction in biomass, number of siliques per plant and number of seeds per silique, especially in plants grown at high density. The simulated removal of 50% of the siliques per plant was partially compensated by an increase in yield per silique. These results confirm that the amount of assimilates available at flowering is the primary determinant of plant yield as found in a previous study in oilseed rape (Wang et al., 2011; Zhang and Flottmann, 2018).

By evaluating plant source and sink formation, and their interaction with plant architectural traits in response to environmental factors and agronomic practices, this thesis provided important information to improve the understanding of the mechanisms underlying yield formation and to define potential breeding targets for improving seed yield in oilseed rape.

6.1.2. Potential applications in breeding

Between the years 1991 and 2010, the oilseed yield in countries such as China, Canada, India, France and Germany grew at annual rates in a range between 0.6 and 2% (Kirkegaard et al., 2016b). However, evidences of a stagnation in yield gains in countries such as the USA, the UK, Finland and Brazil (Peltonen-Sainio et al., 2007a; Rondanini et al., 2012) suggest that new strategies are urgently needed to overcome such levelling-off in yield progress and to satisfy the growing demand for oilseed rape. The most feasible ways to continue increasing crop production of oilseed rape in the coming decades is by plant breeding and by optimizing agronomic practices (Beddington, 2010; Rondanini et al., 2012).

Identifying plant traits associated with high agronomic performance is the starting point to develop high-yielding cultivars and to optimize agronomic practices in agriculture. Optimizing light distribution inside the canopy has been proposed as a potential breeding strategy to improve seed yield in oilseed rape. It has been hypothesized that improving light interception by green leaves during the reproductive stage by, for example, reducing the flower petals size and having more erect and clustered siliques can contribute to increased seed yield (Habekotté, 1997). Simulations performed in Chapter 5 suggest that biomass gains due to improved light penetration inside the canopy are only marginal, without significant effects on seed yield. Indeed, improving the light distribution inside the canopy after flowering increases source availability, but apparently oilseed rape is not completely source-limited at that stage. Thus, if source availability is improved at an earlier stage in the plant life cycle (i.e. vegetative stage), it could have a higher impact on plant biomass, increasing plant C status at flowering and seed yield.

The number of seeds per unit area and the single seed weight are two of the main yield components of oilseed rape, and the latest to be set during the plant life cycle (Mitchell, 1970). As described in Chapters 3 and 4, in the previous three decades the primary focus for yield improvements in oilseed rape was through increasing the number of seeds per unit area (Diepenbrock, 2000; Gomez and Miralles, 2011; Peltonen-Sainio et al., 2007b), while average seed weight did not change (Peltonen-Sainio et al., 2007b). Reductions in the number of seeds per unit area, induced by shading at flowering, were fully compensated by an increase in 1000-seed weight in a range between 47 to 61% in experiments performed in two different years in Chile (Chapter 4). This source limitation increased the seed yield per silique up to twofold the original value in siliques positioned at the bottom of the branches and without negative effects on concentrations of oil and protein, the main target compounds for oilseed growers and breeders. Based on these results, it was proposed that the plasticity in single seed weight can be targeted as a promising tool for improving 1000-seed weight and seed yield per unit area, without negative effects on quality traits. But to achieve this complex objective, physiological and genetic bases of the higher potential single seed weight involved in the current study should be further elucidated. For instance, further research should investigate whether the realization of heavier seeds, in response to the reduction of assimilates at flowering, is triggered by (Chapter 4):

1) a switch in the potential single seed weight,
2) the influx of more assimilates into the individual seeds because of a sink reduction caused by the reduction in number of seeds per silique, when the incoming PAR is again increased to 100%, or
3) non-resource signals.

Genetic gains by overexpressing single seed weight will only be possible if there are no trade-offs between yield components. Quantitative trait loci regulating independently the number of seeds per silique and the single seed weight should be identified, after which the use of Marker-Assisted Selection (MAS) breeding could be a promising tool to increase seed yield per unit area, as proposed for other crops like in cereals (Chen et al., 2016; Gouda et al., 2020) and legumes (Francia et al., 2005).

The identification of potential breeding targets to improve genetic gains in oilseed rape is a promising first step in designing an oilseed rape ideotype. But manipulating plant traits to achieve higher yields or better seed quality is not so straightforward. Indeed, expected advantages of sub-cellular molecular interventions are either dampened or reversed at the plant population level in the field. This happens, because of the multiple feedbacks and trade-offs across different levels of plant organization at both spatial- and temporal scales, and dependence on specific environmental requirements for expression of introduced genes (De Costa, 2018). For this reason, plant breeding techniques should be supported by solid knowledge on the physiological mechanisms underlying plant responses to environmental factors and innovative agronomic practices.

6.1.3. Optimizing agronomic practices

Oilseed rape is a crop characterized by a high requirement of N input and low N-use efficiency (Gammelvind et al., 1996; Schjoerring et al., 1995). Optimizing the balance between N fertilizer input and plant population density, two of the most important agronomic practices in oilseed rape production (Li et al., 2014; Ren et al., 2017), is key for a more efficient use of N fertilizers, while maintaining high yields. In Chapter 2, it was found that leaf area was affected by N supply, with important effects on light distribution and C assimilation at crop level. Indeed, the interaction between N supply and plant population density influences the leaf area index, which determines the proportion of light intercepted by the plant canopy (Kuai et al., 2016). This suggests that canopy light interception and plant carbon assimilation can be optimized by optimizing N fertilizer supply before flowering and plant population density, two of the agronomic practices with the largest impact on seed yield.

When designing strategies for improving oilseed rape productivity, the optimization of plant architectural traits like stem branching, is an important aspect to be taken into consideration (Diepenbrock, 2000). Stem branching is one of the seed yield components most associated with seed yield per unit area and one of the most plastic traits in the oilseed rape crop as observed in Chapter 5. The development of lateral branches is strongly affected by plant population density and N availability. Experiments performed in Argentina with spring oilseed rape demonstrated that reductions in plant density from 240 to 60 plants m^{-2} were fully compensated by the development of more branches per plants and more siliques per plant, without negative effects on seed yield per unit area (Rondanini et al., 2017). On the other hand, reductions in plant population density can also be partially compensated by N input, which stimulates an increase in leaf area index, light interception by the canopy and crop biomass, increasing the number of siliques per plant, yield per silique and yield per unit of area (Kuai et al., 2016).

Simulations performed with the FSP modelling approach (Chapter 3) suggested that without limitations of light, water or nutrients, oilseed rape could almost compensate for a hypothetical reduction in the number of branches per plant (e.g. through branch pruning, or branch inhibition), by increasing the source/sink ratio after budding and extending the period of silique formation. But extending the reproductive stage by a factor four has several constraints for spring oilseed rape growth and development (discussed in Chapter 3), so stem branching is important for maximizing seed yield per unit area, especially under low plant population density, by for example allowing the plant to produce a higher number of siliques in a shorter time than plants with inhibited branching. Further studies should consider quantitative approaches based on plant or crop growth models to evaluate potential interactions between plant population density and N supply and their effect on plant traits associated with seed yield per unit area. This can contribute to the design of more rational fertilization strategies for high and low external input systems.

The use of larger seeds at sowing time improves the seedling establishment, by reducing the period between sowing and emergence, increasing the early vigour of the emerged seedling, and biomass (Harker et al., 2015) and *PAR* interception at flowering (Mendham et al., 1981). In marginal regions where rainfall is an important constraint for crop establishment, larger seeds would allow deeper sowing in soil layers with higher moisture content, improving crop establishment and early vigour (Brill et al., 2016). In addition, larger seeds increase the tolerance to pests such as flea beetles (*Phyllotreta spp.*) (Elliott et al., 2008). All these factors can contribute to reducing the use of agrochemicals for weed and insect control in oilseed rape.

Thus, improving the knowledge about the impact of decreased source-sink ratio on seed yield components and their interactions found in the present study is critical for both plant breeding and agronomic management of oilseed rape.

6.1.4. Combining experimental and modelling approaches to understand oilseed rape plasticity

Identifying and understanding traits associated with high crop performance depends on the quantitative understanding of the extent to which sources or sinks limit plant growth during the plant life cycle (White et al., 2015), and the response of the yield components to agronomic practices. This requires an analytical approach integrating plant organ growth, its response to environmental factors, and a final upscaling to the level of biological organization relevant for agronomic research (i.e. yield per plant and per unit area). In the current study the research questions were addressed through experimental approaches (Chapters 2 and 4) and by combining experimental and modelling approaches (Chapters 3 and 5), based on the hypothesis that the plant C status and plant architectural traits are in constant interaction, determining the plastic response and agronomic performance of oilseed rape.

Modelling offers significant advantages as it allows us to understand how various factors might restrain plastic responses under a wide range of potential scenarios that might be difficult or impossible to manipulate experimentally (Murren et al., 2015). One of these examples is the pioneering work that compares the plastic limits of non-plastic and plastic plants, simulating their performance at different environments (Van Tienderen, 1991). In this thesis, the FSP modelling approach demonstrated to be a useful tool to explore dynamic plant-to-plant interactions in response to agronomic management and source-sink manipulations, particularly for plants with high phenotypic plasticity such as oilseed rape. The acquisition of extensive phenotypic data (phenomics) is now becoming a priority (Kühl and Burghardt, 2013), especially the acquisition of phenotypic data to be used as input parameters for model calibration. To be more specific, parameter requirement is one of the main issues that FSP model users have to overcome (Evers, 2016), because the number of parameters and the type of measurements needed to obtain the parameter values are highly demanding in time and in labour, certainly in comparison with many other simulation models. Nevertheless, if these obstacles can be overcome, FSP modelling offers a wealth of possibilities for oilseed rape research.

Combining modelling approaches with new breeding methods can contribute to improve the efficiency of ideotyping for specific environments (Rötter et al., 2015). This combined approach can be an opportunity for analytical tools like FSP modelling, which can contribute to evaluating the potential effect of increasing source-sink ratio at flowering in oilseed rape by, for example, improving *LMA* or C assimilation during the vegetative stage, or increasing the potential seed weight during reproductive stage. The model can estimate the trait value by considering interactions with other plant traits in response to the local environment, and the results can be scaled up to the crop level. Moreover, the plant phenotype can be visualized in 3-D, which is practical for breeding purposes. The potential of using a modelling approach to assist the genetic analysis of quantitative crop traits whose phenotype is often environment-dependent, has been demonstrated in traits such as flowering phenology in barley (Yin et al., 2005), leaf elongation rate in maize (Reymond et al., 2003) or uptake and root growth and architecture in winter wheat (Laperche et al., 2006), but in all these cases the approach has been done by a process-based model. Therefore, combining breeding with an FSP modelling approach can improve the efficiency of breeding and can accelerate the delivery of future oilseed rape cultivars adapted to changing environments.

Many research questions that deal with crop development, competition, plant/crop physiology, and crop management (i.e. row spacing and density, pruning, spraying of biocides, etc.) require plant architecture to be considered, and therefore can be explored by the FSP modelling approach (Zhu et al., 2015). One of the main problems for oilseed growers are the yield losses caused by plant lodging. The susceptibility to plant lodging is mainly influenced by plant height, stem branching pattern, and the diameter and lignin content of the stem. All these traits are affected by plant population density and N supply (Khan et al., 2018; Kuai et al., 2016). The plant population density and N supply that maximize yield per unit area and minimize risk of lodging can also be simulated by FSP modelling. But for this purpose, plant mechanical properties should be further incorporated into the model.

Combining genomic and phenotypic information, new bioinformatic and statistical tools, and clever experimental design will allow quantitative evaluation of genetic, environmental and epigenetic variations and further insights into the potential constraints to plasticity (Murren et al., 2015). This can contribute to a better understanding of the mechanisms underlying the plant responses to environmental factors, which is the key to develop high-yielding cultivars and to optimize agronomic practices in agriculture.

6.2. Concluding remarks on results

The aim of this thesis was to evaluate the plastic response of oilseed rape to environmental factors and agronomic practices by identifying the main drivers and mechanisms underlying plant C and N and analysing the consequences of C and N distribution on plant architectural traits and potential interactions with traits associated with seed yield. For this purpose, experimental and modelling approaches were combined to address the following research questions:

Q1. To what extent does the interaction between N supply and plant population density alter N distribution and leaf traits in oilseed rape?

Q2: What is the importance of N fertilizer supply in branch and siliques outgrowth?

Q3: Can seed weight compensate for reductions in seed number in response to artificially induced source limitation at flowering?

Q4: Do the seed yield components of oilseed rape differ in their plastic response to N supply, plant density and plant C status at flowering?

The importance of N fertilizer supply on plant N distribution and whether N supply changes plant investment in leaf area or leaf mass per area in oilseed rape was evaluated by an experiment at two rates of contrasting N supplies and two plant population densities.

It was found that leaf mass per unit of leaf area (*LMA*) was affected by a significant interaction between N supply and plant population density. Indeed, discrepancies among previous studies where N availability (DeJong et al., 1989; Jullien et al., 2009; Poorter et al., 2009) or leaf light conditions (Gan et al., 2002; Niinemets, 1997) were identified as the main source of variation of *LMA* could be due to the fact that effects of N and light are hidden by their interaction. But differences among plant species and genotypes used in the studies also need to be taken into consideration when contrasting results. Leaf area was affected by N supply, with important effects on light distribution and C assimilation at crop level before flowering.

Stem branching is one of the most plastic traits in the oilseed rape plant. To evaluate the effect of N supply on branch and silique development, an FSP model was adapted for oilseed rape. Branch and silique outgrowth were implemented in the model as dependent on the plant source/sink ratio status, mainly driven by light interception. Since the number of branches and the number of siliques per plant simulated by the FSP modelling approach were similar to the measured numbers, the hypothesized mechanism that the number of siliques per plant is determined by the source/sink ratio, which in itself is affected by the number of branches, was confirmed. N availability determines bud outgrowth through modifying the C assimilation and thus the source/sink ratio. The modelling approach also showed that the main raceme of an oilseed rape plant has the potential to develop a number of siliques that is similar to that of an entire plant with several branches. However, in real life this cannot be reached due to multiple factors discussed in this thesis.

The paradigm that assumes that the number of seeds per unit area is the main source of variation of seed yield per unit area in response to variations in plant C status at flowering, and that average seed weight is mostly a conserved trait (Sadras, 2007) was evaluated for oilseed rape. For this objective, the plastic response of the number of seeds per unit area, the number of seeds per silique, the 1000-seed weight, the average seed weight and quality traits was evaluated in oilseed rape in two field experiments under i) two plant densities and ii) shading or no shading between the beginning and end of flowering. Reductions in seed number per area in response to shading were due to reductions in both the number of seeds per silique, and the number of siliques per area. Surprisingly, the negative effect of shading on number of seeds per area was fully compensated for by an increase in 1000-seed weight. These results suggest that heavier seeds can be used as a potential strategy to improve seed yield per unit area. To achieve this complex objective, the physiological bases of the higher potential seed weight and its interplay with other seed yield components should be elucidated.

Finally, the evaluation of the sensitivity of the yield components of oilseed rape in response to plant density, N supply and source limitation at flowering showed the following ranking: 1000-seed weight > number of siliques per plant > number of branches per plant > number of seeds per silique. Thousand-seed weight was at the top of this ranking exclusively due to the 60 and 70% heavier seeds observed in response to shading at flowering in two experiments. However, this trait did not respond to any other environmental factor. This suggests that the sensitivity of plant traits to environmental factors is not static and dependent on the crop, the type and timing of source limitation.

The effects of reduced source-sink ratio were simulated by an FSP model. The simulated shading caused a reduction in biomass, number of siliques per plant and number of seeds per silique, especially in plants grown at high density. The simulated removal of 50% of the siliques per plant was partially compensated by an increase in yield per silique. The results obtained by the current modelling approach suggest that the amount of assimilates available at flowering is the primary determinant of plant yield.

FSP modelling offers a wealth of possibilities for oilseed rape research. For example, the potential effects of increasing the source-sink ratio during vegetative stage by optimizing *LMA* and C assimilation during the vegetative stage, or the interplay between number of seeds per silique and single seed weight can be explored by FSP modelling. But also, many research questions related with crop management like for example reducing plant lodging by identifying the optimal plant population density and N supply, can be addressed by an FSP modelling approach.

Identifying and understanding the mechanisms and traits associated with high crop performance depends on the quantitative understanding of the extent to which sources or sinks limit plant growth during the plant life cycle (White et al., 2015), and the response of the yield components to agronomic practices. Combining experimental approaches with analytical tools like for example FSP modelling can contribute to integrate plant organ growth, its response to environmental factors, and a final upscaling to the level of biological organization relevant for agronomic research.

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Summary

Over the last 50 years, the seed production of oilseed rape (*Brassica napus* L.), the source of one of the healthiest edible oils for human consumption, has increased globally, reaching 71 million tonnes in 2017. However, food security will be challenged by the growth of the world's population, reaching 9.7 billion people in the next 50 years, increasing the demand of food, vegetable oils for human consumption, feed and biofuel. Therefore, new strategies are urgently needed to satisfy the growing demand for oilseed rape. Since the expansion of cultivated area does not seem to be a large-scale sustainable strategy, yield improvement by plant breeding and by optimizing agronomic practices appears as the most feasible way to continue increasing crop production in the coming decades and to satisfy the growing demand for oilseed rape. One such strategy revolves around making use of plasticity in oilseed rape.

Phenotypic plasticity is the ability of a genotype to modify its phenotypic expression in response to the local environmental conditions and several studies have demonstrated a high plasticity in oilseed rape in response to the local environment and agronomic practices. Two of the agronomic practices with the highest impact on oilseed rape production are nitrogen (N) fertilizer supply and plant population density, which affect plant N and carbon (C) distributions during the plant's life cycle. In plant canopies, leaf N distribution follows the light distribution, in an exponential decline over the canopy depth, which agrees with the optimal distribution of leaf N to maximize C assimilation and crop productivity. The relationship between leaf N content and photosynthetic rate per unit leaf surface has been investigated in a range of different plant species, but so far not in crops like oilseed rape.

Another important factor affecting light distribution is the stem branching pattern, but branching is itself determined by light. This is the case as the number of branches per plant depends on the number of axillary buds along the stem that break their dormancy. It is known that bud outgrowth is influenced by several internal and external factors, among which photosynthetic active radiation (PAR), plant C status, red: far red ratio, sugar content and their interaction with the concentrations of hormones such as auxins, strigolactones and cytokinins, have been identified as the main ones responsible for bud outgrowth and subsequently for stem branching. It is also known that N is associated with C assimilation and meristematic activities, but there is no conclusive evidence of a direct role of N availability on bud outgrowth. Since

the number and spatial distribution of stem branches determine plant architecture and plant source/sink ratio, and the number of seeds per silique, the agronomic performance of oilseed rape could be determined by the interaction between stem branching and plant source/sink ratio.

Oilseed rape can adapt its phenotype in response to resource availability and environmental signals from early developmental stages by, for example, modifying leaf and petiole size or, even later at flowering, by adjusting the number of siliques per plant, or the number of seeds per silique. It is assumed that resource limitations have a negative effect on seed yield components, but the quantitative responses of the seed yield components, such as the number of seeds per unit area, single and average seed weight, and seed quality traits to source limitation at flowering have been scarcely assessed in oilseed rape.

Identifying the main determinants of source and sink formation, and their interaction with plant architectural traits, can provide important information to improve the understanding of the mechanisms underlying yield formation and to define potential breeding targets for improving seed yield in oilseed rape. Therefore, this thesis evaluated the plastic response of oilseed rape to environmental factors and agronomic practices by identifying the main drivers and mechanisms underlying plant C and N distribution, with emphasis on the interaction between plant C status with plant architectural traits and physiological traits associated with seed yield. For this purpose, experimental and modelling approaches were combined to answer the following research questions:

Q1. To what extent does the interaction between N supply and plant population density alter N distribution and leaf traits in oilseed rape?
Q2: What is the importance of N fertilizer supply in branch and siliques outgrowth?
Q3: Can seed weight compensate for reductions in seed number in response to artificially induced source limitation at flowering?
Q4: Do the seed yield components of oilseed rape differ in their plastic response to N supply, plant density and plant C status at flowering?

To what extent does the interaction between N supply and plant population density alter N distribution and leaf traits in oilseed rape?

Chapter 2 evaluated the importance of N fertilizer supply on plant N distribution and assessed whether N supply changes plant investment in leaf area or leaf mass per area in oilseed rape. Spring oilseed rape was grown at two rates of N supply (50 and 150 kg ha^{-1}) and two plant population densities (50 and 150 plants m^{-2}) in containers, under an open-sided shelter. Traits such as photosynthesis, leaf area, biomass and N content of selected leaves were measured at budding stage. N supply determined N content per unit of leaf mass (N_{mass}), while it played a subordinate role in altering N per unit of area (N_{area}), which was the main determinant of C assimilation. The rate of photosynthesis per unit of leaf area was strongly correlated with N content per leaf area (N_{area}) through an asymptotical relationship. N_{area} was more closely associated with leaf mass per unit of leaf area (*LMA*) than with N_{mass}. *LMA* was affected by a significant interaction between N supply and plant population density. Such an interaction plays an important role in the discrepancies observed in literature. Therefore, differences in *LMA* observed in spring oilseed rape during a specific phenological stage are not totally comparable with those observed in studies performed in other plant/crops or in spring oilseed rape at different phenological stages.

Leaf area was affected by N supply, with important effects on light distribution and C assimilation at crop level. Indeed, the interaction between N supply and plant population density influences the leaf area index, which determines the proportion of light intercepted by the plant canopy. This can have an important effect on the amount of light intercepted by the canopy and associated plant carbon assimilation before flowering, which is the foundation of crop yield.

What is the importance of N fertilizer supply in branch and siliques outgrowth?

In Chapter 3, the role of N supply as determinant of plant source-sink status, which is, in turn, the main determinant of branch outgrowth and silique production, was evaluated by combining experimental and Functional-Structural Plant (FSP) modelling approaches. The results obtained in the experiment revealed significant effects of N fertilizer supply on plant C assimilation, with maximum leaf and silique photosynthesis being 16% and 87% higher, respectively, at high N supply than at low N. This was rather expected as N supply has been found to be the major factor affecting C assimilation in several plant species. To evaluate the role of N supply on branch outgrowth, an FSP model was adapted for oilseed rape. Branch and silique outgrowth were implemented as dependent on the plant source/sink ratio status, mainly driven by light interception. Since the numbers of branches and siliques per plant simulated by the FSP modelling approach were similar to the measured numbers, the hypothesized mechanism that the number of siliques is determined by the source/sink ratio, which in itself is affected by the number of branches, was confirmed. The relationship between the number of branches per plant and the source/sink ratio at budding revealed that next to the effect of N on C assimilation, an additional factor or factors that change(s) with population density are likely to be involved in determining bud outgrowth, not considered in this study. Additionally, the hypothetical limits of oilseed rape plants to produce siliques under a simulated branch inhibition were also explored in Chapter 3. The objective of this exercise was to evaluate the importance of stem branching in plant productivity, considering number of siliques per plant as a proxy of plant yield. For this purpose, the number of siliques produced by plants with only one branch (the main raceme) was compared with the number of siliques of a plant with multiple branches. The results showed that the main raceme of an oilseed rape plant has the potential to develop a similar number of siliques as a plant with several branches. However, to achieve this full compensation, the model assumed several phenomena that are not very realistic, discussed in Chapter 3 and highlighting the importance of branching on yield.

Can seed weight compensate for reductions in seed number in response to artificially induced source limitation at flowering?

Chapter 4 challenged the paradigm that assumes that seed number per unit area is the main source of variation of seed yield in response to variations in plant C status at flowering, and that single seed weight is mostly a conserved trait. Thus, the following research questions were addressed: i) do seed number per unit area and single seed weight in oilseed rape differ in their plasticity in response to the availability of assimilates at flowering? and ii) how sensitive are oil and protein concentrations to the availability of assimilates during flowering? To answer these questions, the plastic response of seed number per unit area, single seed weight and quality traits were evaluated in oilseed rape in two field experiments under i) two plant densities and ii) shading or no shading between the beginning and end of flowering.

Seed yield per area did not differ when plants were grown at 40 or 80 plants m^{-2} (Exp. 1), or at 30 or 45 plants m^{-2} (Exp. 2), neither did it change in response to shading at flowering. These findings demonstrate the remarkable compensatory capacity of oilseed rape. At low plant density, oilseed rape plants had higher biomass per individual plant, more branches and siliques per plant and, ultimately, more seeds per plant, while 1000-seed weight was highly conserved and did not respond to plant density, which is in line with previous studies in oilseed rape.

Reductions in seed number per area in response to shading were due to reductions in both the number of seeds per silique, and the number of siliques per area. Surprisingly, the negative effect of shading on seed number per area was fully compensated by an increase in thousand seed weight ranging from 47 to 61% without affect oil and protein concentration. This increase in single seed weight was largely a result of both a higher rate of seed filling and a longer duration of seed filling across all seed positions in the canopy. Recently, other authors also explored the plastic response of oilseed rape in response to source-sink manipulations, finding increases in single and average seed weight, but not in the magnitude found in this thesis and only partially compensating for reductions in seed number per unit area. This chapter hints at the necessity of re-evaluating the paradigm assuming that seed number per unit area is the main source of variation of seed yield, while seed weight is mostly a conserved trait, and for modelling purposes can be considered constant. Indeed, how stable or plastic a single plant trait is, depends on the availability of resources, and on environmental signals, but also on the moment at which resources are available.

Do the seed yield components of oilseed rape differ in their plastic response to N supply, plant density and plant C status at flowering?

Chapter 5 evaluated the sensitivity of the main seed yield components that account for variation in seed yield of oilseed rape in response to different environmental factors, using datasets collected from experiments performed in the Netherlands and Chile. Plastic responses of seed yield to source/sink ratio manipulations at flowering were quantified using an FSP modelling approach.

Variations in seed yield per unit area were mostly explained by the number of seeds per unit area, while seed yield per plant strongly associated with variations in the number of branches per plant, number of siliques per plant and number of seeds per plant, while yield per silique was explained by the number of seeds per silique. But this does not mean that single seed weight is a conserved trait, taking into account that under source limitation at flowering single seed weight was increased up to twofold compared with the treatments under full sunlight. Indeed, when comparing the stability of the yield components of oilseed rape in response to source limitation at flowering, single seed weight was classified as the most plastic trait. However, this was the most stable trait in response to all the other environmental factors evaluated in this thesis (i.e. plant density, N supply, weather conditions in the different experiments). This suggests that the sensitivity of plant traits to environmental factors is not static and dependent on the crop, the type and timing of source limitation.

In the same chapter, different plant source/sink ratios were simulated by an FSP model previously adapted in Chapter 3 for oilseed rape. The simulated shading caused a reduction in biomass, number of siliques per plant and number of seeds per silique, especially in plants grown at high density. The simulated removal of 50% of the siliques per plant was partially compensated by an increase in yield per silique. These results confirm that the amount of assimilates available at flowering is the primary determinant of plant yield as found in a previous study in oilseed rape.

Finally, in Chapter 6 the key findings and approaches of this thesis were further evaluated for their merits in answering crop physiological and agronomic research questions.

By evaluating plant source and sink formation, and their interaction with plant architectural traits in response to environmental factors and agronomic practices, this thesis provided important information to improve the understanding of the mechanisms underlying yield formation and to define potential breeding targets for improving seed yield in oilseed rape. Combining experimental approaches with analytical tools like for example FSP modelling can contribute to integrate plant organ growth, its response to environmental factors, and a final upscaling to the level of biological organization relevant for agronomic research.

Acknowledgements

"Men wanted for hazardous journey. Low wages, bitter cold, long hours of complete darkness. Safe return doubtful. Honour and recognition in event of success". This advertisement was posted in 1913 by Ernest Shackleton, an Anglo-Irish Antarctic explorer, to select candidates for his expedition to the South Pole. Here I am, almost a hundred years later with the satisfaction of being at the end of my journey as PhD candidate, a process which combined some of the elements described by Shackleton in his advertisement with plenty of beautiful moments.

Many people contributed directly or indirectly to finish this journey successfully, to whom I would like to express my sincere gratefulness. Firstly, I would like to thank my supervisors Paul Struik, Jochem Evers and Daniel Calderini. Dear Paul, many thanks for your support, patience and excellent guidance during my process as PhD candidate. Your attention to detail and ability to detect errors contributed to improving the quality of this thesis. I have been very lucky for having you as promotor. Dear Jochem, thank you very much for guiding me during my process as PhD candidate. Your analytical skills, clarity of mind and capacity on how to put ideas in a logical order, were key factors to succeed and finish my thesis. I appreciate your patience and the time invested on me. Dear Daniel, you guided me during my bachelor, MSc, and PhD processes. Thank you for your academic and personal support, which most of the time went beyond your responsibilities as supervisor (i.e. issues with my scholarship and many other situations). I really appreciate your and Magda's trust and friendship during all these years. I'm looking forward to continuing working together in future projects.

I will make special mention of three colleagues without whom I doubt I would have succeeded. Peter van der Putten, Gou Fang and Hiroe Yoshida, thank you very much for your support in my experiments, but also for your friendship. We had a great time working together, sharing coffee breaks, and organizing drinks and parties in Wageningen. I would like to also thank Jeroen de Bruijn and Jin W. Kim, who did their MSc internship on my project and made an important contribution to my thesis. To Georgios Angelopoulos, Niel Verhoog, UNIFARM, Constanza Vargas, Victoria Abarzúa and the experimental station of the Universidad Austral de Chile, for their technical support in my experiments in the Netherlands and Chile.

I am also grateful to Olaf Sass (NPZ-Lembke, Germany), Erik von Baer and to my dear friend

Julliette Navarrete (Semillas Baer, Chile) for providing me with the seed for all my experiments and to Edmundo Klein (Bayer S.A.) for kindly providing me with the agrochemicals needed for my experiments in Chile.

I am very grateful to Jorad de Vries, for helping me to understand the FSP modelling approach and for his contribution to adapting the FSP model framework to oilseed rape, also to Dr Jan Vos and to Dr Xinyou Yin for the nice discussions and suggestions on my thesis. I would also like to thank many PhD fellows and friends: Luuk van Dijk, Alejandro Morales Sierra, Giovani Theisen, Dennis Tippe, Ali Elhakeem, Ouyang Wenjing, Uta Priegnitz, Martin Sikma, Kailei Tang, Naznin Nahar, Gou Fang, Herman Berghuijs, Gu Shenghao, Arian van Westreenen, Maarten van der Meer, Marloes van Loon, Jorad de Vries, Yu Yang, Cesar Ospina, Ambra Tosto, Ioannis Baltzakis, Chen Bin, Junqi Zhu, André Braga Junqueira, Marcia de Melo Carvalho, Franca Bongers, Ningyi Zhang, Li Guohua, Merel Jansen, Stella Kabiri, Niteen Kadam, Joao Nunes Vieira da Silva and Rachel Schipper. Thank you very much for organizing scientific seminars, PhD meetings, lunch discussions, international dinners, Thursday drinks, and outdoor activities. Luuk and Alejandro, thank you very much for willing to be my paranymphs and organizing my defence ceremony and party.

Thanks to the staff members of the Centre for Crop Systems Analysis (CSA), especially Nicole Wolffensperger and Petra Rozema, but also the former CSA members Sjanie van Roekel and Alex-Jan de Leeuw. I appreciate your kindness and dedication helping us to sort out many little details, while making us feel at home at the same time. Also, to Lammert Bastiaans and Wopke van der Werf, with whom I had nice conversations during my years at CSA.

There were also some other friends from outside CSA that were very important to me in the Netherlands and Belgium. Thank you, Rafael Chan, Marcela Víquez, Marcos Malosetti, Roxina Soler, Martin Boer, Julio Velazco, Vincent Garin, Vicky Alomia, Pieter Van 't Hof, Pablo Modernel Eduardo Zerene, Héloïse Giraud and Joris de Wolf, for making my journey a nice experience, helping me to get around and for sharing your experiences.

To the Chilean-WUR community in Wageningen for providing me support, encouragement, and for organizing social events that made me feel like home. Especially to Pame Bachmann, Diego García-Mendoza, Marcia Bodero, Gerlof, Niko, Grace, Mauricio, Loreto, Henk, Roberto

Chávez, Marcela Riquelme, Andres Schlageter, Fran, Julian, Luis, Yelica, Daniel, Lucía, Diego Safián, Maite, Jose, Rodolfo, Felipe L, Felipe B, Seba Decap, Jo, Carlos, Ale, Fran, Roro, Fabio, Maca, Pablo R, Naty, to my friends Kurt Ruf, Pepe Correa and my sister in law Mariana Bustos. Also, to Daniela's family in Chile, Marianne, Juan de Dios, María Paz, Paulina, aunts, Opa and Oma thanks a lot for your support. A special thanks to Begoña Arellano for designing the cover of this thesis.

I want to thank Aphea.Bio, my current team. Dear Isabel and Steven, thank you for your trust in me. A special thanks to my colleagues Behnoush, Elke, Jonas and Tibby, for encouraging me during the last stretch of my PhD. Also, to Hanna, Kelly, Katrien, Tom, Mohamad, Michael, Oliver, Dries, Frederick, Emma, Jens, Abou, Thomas, Marianita, Katlijn and Waldo. Together we will rock biologicals in Europe!

Mis queridos Carlos, Elisia y Pamelita, ustedes son lo mejor y los más lindos del mundo. No saben lo orgulloso y afortunado que me siento de haber nacido en Panguipulli y ser parte del yepu. Gracias por quererme, guiarme y educarme en cosas que no se enseñan en una universidad. Esta tesis es producto de lo que ustedes, mi tío Luis y la parcela "El Roble" me han enseñado.

Last but not least, I would like to express my deepest gratitude to Daniela Bustos-Korts, my beloved wife. Danielita, you are not only my support in life but during my PhD, you also had an important role as my "non-official supervisor" always giving me your clever opinion on how to improve the quality of my thesis. It is my greatest happiness having you at my side during our journey in Europe, and I am looking forward to us setting forth on our next adventure.

List of Publications

Asseng S., Kassie B.T., Labra M.H., Amador C., Calderini D.F. (2017) Simulating the impact of source-sink manipulations in wheat. Field Crops Res. 202:47-56.

Labra M.H., Struik P.C., Evers J.B., Calderini D.F. (2017) Plasticity of seed weight compensates reductions in seed number of oilseed rape in response to shading at flowering. Eur. J. Agron. 84:113-124.

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)

- Phenotypic plasticity of rapeseed (*Brassica napus* L.) in response to N supply (2014)
- Exploiting phenotypic plasticity of oilseed rape (*Brassica napus*) to reduce nitrogen fertilization (2016)

Writing of project proposal (4.5 ECTS)

- Effects of N availability and plant population density on crop architecture of oilseed rape (*Brassica napus*) and their influence on grain yield components (2013)

Post-graduate courses (3.6 ECTS)

- Introduction to R for statistical analysis; PE&RC (2013)
- Ecophysiology of crops and fruits; Graduate School, Universidad Austral de Chile (2015)
- Modelling plant form and function using GroIMP; PE&RC (2016)

Laboratory training and working visits (0.3 ECTS)

- Crop physiology, modelling; Institut National de la Recherche Agronomique, Paris, France (2014)

Deficiency, refresh, brush-up courses (3 ECTS)

- System analysis, simulation, and system management; WUR (2013)
- Designing sustainable cropping systems; CSA (2014)

Competence strengthening / skills courses (1.5 ECTS)

- Data management; WUR (2014)
- Techniques for writing and presenting a scientific paper; University of Illinois (2016)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.5 ECTS)

- PE&RC Introduction weekend (2013)
- PE&RC Day (2013, 2015)

Discussion groups / local seminars / other scientific meetings (5.5 ECTS)

- Symposium: how to write a world-class paper (2013)
- Sustainable intensification of agricultural systems (2013-2016)
- Plant form and function (2013-2016)
- Symposium of the 96th Dies Natalis: changing environments (2014)
- Symposium measuring the photosynthetic phenome (2014)
- Symposium of the 98th Dies Natalis: the digitalisation of nature (2016)
- Whealbi workshop: databases, SNP calling and genetic analysis of multi- environment data (2017)

International symposia, workshops and conferences (5.4 ECTS)

- 14th International rapeseed congress; poster presentation; Saskatoon, Canada (2015)
- 66th Congreso Agronomico de Chile; poster presentation; Valdivia, Chile (2015)
- Workshop: modelling plant form and function using GroIMP; oral presentation; Wageningen, the Netherlands (2016)

Supervision of MSc students (6 ECTS)

- Nitrogen distribution among pods in oilseed rape (*Brassica napus* L.): the effect of pod appearance and position on pod photosynthetic capacity and grain yield components
- Silique photosynthesis of oilseed rape (*Brassica napus* L.) in response to nitrogen supply and plant population density

About the author

Marcelo Labra was born on the 29th of July 1978 in Panguipulli, Chile. After obtaining his degree of Agronomist in Universidad Austral de Chile, Valdivia, Marcelo worked as research assistant in the Institute of Plant Production and Protection, Universidad Austral de Chile, and two years later, he started his MSc. in Plant Sciences with specialization in Crop Physiology in Universidad Austral de Chile. During this period, he was part of the team of the EU-RHIBAC project “Evaluating the use of plant growth-promoting rhizobacteria to complement the use of fertilizer in wheat”, evaluating the effects of beneficial microorganisms on plant growth, yield and quality traits of wheat. After his MSc study, Marcelo worked as agronomist in Campex Baer, a Chilean breeding company, where he was in charge of testing the performance of crop varieties in multi-environment trials. At the end of 2012, he was awarded with a scholarship from the Comisión Nacional de Ciencia y Tecnología (CONICYT, Chile) to start a PhD with the Centre for Crop Systems Analysis at Wageningen University and Institute of Plant Production and Protection, Universidad Austral de Chile. His research evaluated the phenotypic plasticity of oilseed rape in response to nitrogen supply and plant population density, combining experimental and functional structural plant modelling approaches. From 2017, Marcelo has been the Field Trial Manager of Aphea.Bio, a Belgian company focused on the development of a new generation of biological products for agriculture based on beneficial microorganisms. In parallel, he is co-researcher of the granted project “Physiological mechanisms and key genes associated with the regulation of kernel weight-grain number interaction and temperature response in rapeseed (*Brassica napus* L.)”, with Universidad Austral de Chile.

Funding

The research described in this thesis was funded by the Becas Chile Programme (CONICYT, Chile) and the Graduate School, Faculty of Agricultural Science, Universidad Austral de Chile. Financial support from the Centre for Crop Systems Analysis (CSA) and Wageningen University for printing this thesis is gratefully acknowledged.

Cover design by Begoña Arellano Jaimerena
This thesis was printed by GVO drukkers & vormgevers B.V, Ede, the Netherlands