Crucifer glucosinolate production in legume-crucifer cover crop mixtures

Antoine Couëdel[a,*], Lionel Alletto[a,b], John Kirkegaard[c], Éric Justes[a,1]

[a] AGIR, Université de Toulouse, INRA, INPT, INP-EI PURPAN, Castanet-Tolosan, France
[b] Chambre Régionale d'Agriculture Occitanie, 31321 Castanet-Tolosan, France
[c] CSIRO Agriculture and Food, GPO Box 1700, Canberra, ACT, 2601, Australia

ABSTRACT

Cover crops grown in rotation with cash crops provide ecosystem services by reducing water pollution and anthropogenic inputs. Bispecific crucifer–legume cover crop mixtures are seen as a solution to increase biodiversity and to combine ecosystem services of both species. Legumes fix nitrogen while crucifers have the capacity to suppress pathogens due to the biocidal hydrolysis products of the endogenous secondary metabolites called glucosinolates (GSL). However there is a lack of information on the impact of plant–plant interactions in the crucifer-legume mixtures on crucifer GSL production compared to sole crop. The aim of our study was to assess GSL production of a wide range of bispecific crucifer-legume mixtures in comparison to sole crops. Experiments were conducted at two sites (near Toulouse and Orléans, France) over two years. Various cultivars from eight crucifer (Brassicaceae) species (rape, white mustard, Indian mustard, Ethiopian mustard, turnip, turnip rape, radish and rocket) and nine legume (Fabaceae) species (Egyptian clover, crimson clover, common vetch, purple vetch, hairy vetch, pea, soya bean, faba bean, and white lupin) were tested in sole crop and bispecific mixtures (substitutive design of 50%–50% sole crops). We show that for a wide range of species, crucifers in cover crop mixtures had the same GSL types, concentrations and proportions (aliphatic, aromatic and indole) as in sole crops. Crucifers in mixtures tended to have more biomass per plant, both for shoots and roots, than in sole crops so that the total GSL production per plant generally increased in the mixture compared to the sole crop. Thus despite halving the crucifer density in the mixtures the GSL production on an area basis declined by only 20%. These results have been validated for a wide range of crucifer species and provide support for crucifer-legume mixtures to produce nutrient-related services while potentially maintaining high GSL production. Specific experiments are needed to evaluate whether this double effect of unchanged GSL concentration and small reduction in GSL production has an impact on the biofumigation potential of cover crop mixtures compared to sole crucifers.

1. Introduction

Biodiversity based agriculture has been promoted as one tool to enhance ecosystem services while reducing disservices engendered by anthropogenic inputs (Duru et al., 2015; Garcia et al., 2018). Cover crops grown during the autumn-winter period between two cash crops in annual crop sequences can provide a number of ecosystem-services including improved soil and water quality (Blanco-Canqui et al., 2015; Dabney et al., 2001), nutrient cycling (Thorup-Kristensen et al., 2003) and suppression of weeds (Teasdale, 1996), pests (Farooq et al., 2011) and diseases (Snapp et al., 2005). Among the species available, Brassicaceae or crucifer cover crops have an additional and unique capacity to suppress pests and diseases that is related to the bioactive hydrolysis products of the family of compounds known as glucosinolates (GSLs)

(Brown and Morra, 1997; Van Dam et al., 2009). GSLs are sulfur (S) and nitrogen (N) containing beta-thioglucoside secondary metabolites present in the plant vacuole (Agerbirk and Olsen, 2012). When plant tissues are disrupted, GSLs react with endogenous enzymatic myrosinases to produce numerous biocidal compounds such as isothiocyanates, thiocyanates and nitriles (Brown and Morra, 1997). Intact GSLs released from roots into the soil environment may also be hydrolysed by microbial myrosinases to generate the same products exogenously in the rhizosphere (Gimsing and Kirkegaard, 2009). The types and concentrations of GSLs vary significantly among crucifer species, and between root and shoot tissues of the same species (Bellostas et al., 2007; Kirkegaard and Sarwar, 1998) and the ecological and agronomic implications of GSL interactions with pathogens have been considered in several reviews (Brown and Morra, 1997; Van Dam et al., 2009). The

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interaction of crucifer cover-crop derived GSLs with living organisms in the environment can occur 1) during the crucifer cover crop growing period when GSL and hydrolysis products are permanently released in the rhizosphere (McCully et al., 2008; Rumberger and Marschner, 2004), but also 2) when the cover crop is terminated and the biomass incorporated as either brown (killed by herbicide) or green manure. Evidence for biocidal effects both during growth and after termination of cover crops have been demonstrated (Motisi et al., 2009a, 2010) although much higher concentrations of GSLs and hydrolysis products from both shoot and root tissues have been measured following incorporation (Gimsing and Kirkegaard, 2009). The suppression of pests and pathogens associated with these compounds has been termed "biofumigation" (Matthiessen and Kirkegaard, 2006) and has generated interest from an ecological and agronomic perspective in the regulation of soil biological communities (Bressan et al., 2013), including on nematodes (Potter et al., 1998), fungi (Motisi et al., 2009a), bacteria (Arthy et al., 2002) and weeds (Haramoto and Gallandt, 2004; Jabran et al., 2015). Strategies to capture the beneficial effects of pathogen suppression without negative impacts on beneficial or non-target organisms has been an area of recent interest (Hossain et al., 2015; Omirou et al., 2011).

The biofumigation potential of crucifer cover crops depends significantly on the GSL types and concentration in the plant (Matthiessen and Kirkegaard, 2006) which not only differs between species, but also with environment and agronomic practices (Björkman et al., 2011). Typically crucifer cover crops are grown as pure/sole crops (SC) with the aim of maximizing the biomass and GSL concentration and thereby the GSL production to enhance biofumigation potential. Recently there has been a greater focus on legume and non-legume cover-crop mixtures to enhance the multiple eco-services that may arise from ecological interactions (Finney et al., 2016; Justes et al., 2012). Often when deciding a strategy for cover crops, the highest priority is given to enhanced N-cycling eco-services, either by provision of fixed N or the capture and storage of potentially leachable N. Sole cover crop crucifers have high catch crop capacity but engender lower N mineralisation than sole cover crop legumes (Couëdel et al., 2018). Crucifer-legume bispecific mixtures (i.e. in substitutive design, such as 50% density of each species of the sole crop in the mixture) have been found to improve the amount of N mineralised for the next cash crop compared to crucifer sole crops (Tribouillois et al., 2016). Indeed legumes provide a high input of nitrogen in the agroecosystem due to the high amount of N acquired from the soil and atmosphere (Thorup-Kristensen et al., 2003). Overall non legume–legume mixtures have been shown to produce more biomass than non-legume sole crops because of the N_2 fixation of legume increasing N inputs and also due to their better resource use efficiency, due to the niche complementarity in using abiotic resources such as light, water and nutrients (Jensen, 1996). Non-legume − legume mixtures have also been demonstrated to provide better suppression of certain pests, diseases and weeds compared to sole crops (Trenbath, 1993; Boudreau, 2013).

Despite the significant interest in pest-suppressive and catch-crop capacity of crucifer cover crops, and the concurrent interest in the potential of bispecific mixtures, there have been few studies investigating the impact of inclusion in crop mixtures on GSL profiles and production (amount of GSL produced per unit of surface) of the crucifer component. A number of different plant–plant interactions in bispecific mixtures could be anticipated to influence the types and concentrations of GSL and the level of production of crucifers and thereby the biofumigation effect such as pest-suppressive eco-services of the mixtures. For example, in mixtures with legumes, crucifer cover crops are usually in competition for water, nutrients and light due to their rapid root and shoot growth (Kristensen and Thorup-Kristensen, 2004). As a result, on an individual plant basis, crucifers have been shown to produce a higher biomass (Wortman et al., 2012), to acquire more N (Couëdel et al., 2018; Tribouillois et al., 2016; Wendling et al., 2017) and more S (Génard et al., 2017; Couëdel et al., in press) when mixed with a legume

crop than in a sole crop. As N and S are both essential components of GSLs, a different GSL production per crucifer plant could therefore be expected in mixtures compared to sole crops. Indeed in sole crops, an increasing N and S supply by fertilisation has been shown to generate a higher GSL production per plant in crucifers (Falk et al., 2007; Li et al., 2007; Omirou et al., 2009). These competitive and interactive effects could influence mechanisms of GSL production (per plant and per unit of ground area cropped) and release from growing roots or shoots during cover-crop growth, and the final total GSL production available at incorporation. At the plant level, changes in GSL may influence crucifer susceptibility to pests and diseases while the biofumigation potential of the cover crop would also be influenced by changes on an area basis. In the only two studies available investigating impacts of bispecific mixtures on GSL production, Stavridou et al. (2012) found no impact on broccoli (*Brassica oleracea*) in a substitutive design mixture (50%-50% density of each of sole crop) with lettuce (*Lactuca sativa*), while Björkman et al. (2008) found that both shoot and root GSLs in cabbage (*Brassica oleracea*) decreased when mixed with clover in an additive design (100%–100%, i.e, at the same density of each of sole crop). To our knowledge there have been no studies conducted to assess the effect of crucifer-legume mixtures on GSL production in a substitutive design under cover crops grown in arable cropping systems (field conditions).

We report a study designed to assess the impacts of crucifer-legume bispecific mixtures (50%-50% substitutive design) on the GSL type, concentration and production at both the individual plant level and on a ground area basis. The capacity of crucifers to strongly compete with legumes for water, N and S acquisition prompted us to test two hypotheses:

1) Crucifers in bispecific cover crop mixtures with legumes will have the same total GSL concentration as in the sole crop
2) Bispecific mixtures will produce more than 50% of sole crop GSL on a ground-area basis due to increased biomass production per crucifer plant with no reduction in GSL concentration.

Recent studies have highlighted that climate, soil type and N availability at sowing have the greatest influence on cover crop mixture performances (Tribouillois et al., 2016). Accordingly the experiments reported here included a range of different crucifer and legume species and varieties in the mixtures and were conducted over two years at two contrasting sites from late summer to late autumn to assess the generality of species mixture interactions.

2. Materials and methods

2.1. Experimental design and cover crop management

Four field experiments were conducted over two years (2014 and 2015) at two sites: 1) the Lamothe experimental farm of INP-EI Purpan, located in Seysses, 20 km south of Toulouse, south-western France (43.506°N, 1.237°E), and 2) the La Vannelière research station of Jouffray Drillaud, located 50 km south-east of Orléans, central France (47,776°N, 2.098°E). Experiments conducted in 2014 and 2015 are referred to as L2014 and L2015, respectively, at Lamothe (L) and as V2014 and V2015, respectively, at La Vannelière (V). According to the Köppen climate classification, Lamothe (Toulouse) has a humid subtropical climate and La Vannelière (Orléans) has an oceanic climate (Table 1). At all four site-years, the experiment was a completely randomised design that was replicated with three replicates in blocks. Individual sub-plots were arranged with 10 rows at 15 cm spacing for each treatment, with plot area of 18 m^2 for L2014 and V2014, 22.5 m^2 for L2015 and 20.0 m^2 for V2015. To avoid plant–plant competition effects between adjacent treatments, only the six central rows in each plot were sampled for soil and plant measurements.

A range of cover crop species were selected for their ability to grow