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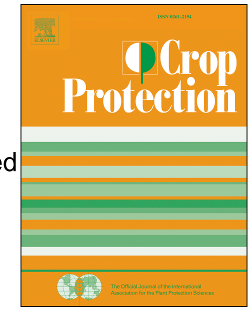

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PII: S0261-2194(26)00259-0

DOI: https://doi.org/10.1016/j.cropro.2026.107793

Reference: JCRP 107793

To appear in: *Crop Protection*

Received Date: 11 February 2026

Revised Date: 22 July 2026

Accepted Date: 28 July 2026

Article type: Short communication

Words abstract: 229

Words body text and references but excluding tables and figures: 2,845

Pages: 19

Tables: 2

Figures: 2

Supporting information: 6 tables

Perennial herbaceous crops for bioenergy and biorefining can play an important weed management role in IPM programmes

by

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Short title: **Germinable weed seed bank development in biocrops**

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Abstract

Crops for bioenergy and biorefining purposes (termed 'biocrops') may become more widespread in future arable cropping systems alongside the development of biomass processing technologies. Some perennial biocrops can be grown without chemical herbicides and can, consequently, contribute to reducing herbicide input in integrated pest management (IPM) programmes at the farm level, depending on the area and duration of biocrops. The objective was to clarify whether perennial biocrops result in germinable weed seed banks greater than those found with annual biocrops receiving chemical herbicides. This could counteract weed management in subsequent crops. Soil samples were taken at different times in two long-term experiments with cropping systems for both annual and perennial biocrops in Denmark. Continuous grass clover and perennial grass (tall fescue) generally had the smallest germinable seed banks with no increase in seed bank sizes after five and seven years of cropping respectively. Annual biocrops also had small germinable seed banks, but chemical herbicides were used annually to control weeds in contrast to grass clover and tall fescue receiving no pesticides. The lignocellulosic perennial biocrops, willow and *Miscanthus* x *giganteus*, had by far the greatest germinable weed seed banks, mainly because of insufficient weed suppression during early establishment and after cutting events. Herbaceous perennial biocrops creating a dense and competitive plant cover that regrows quickly after several cuttings per year seem most promising for weed suppression in IPM programmes.

Keywords: grass clover, tall fescue, miscanthus, willow, germinable weed seed bank, cropping system

Herbaceous perennial crops, such as grass clover, grasses and lucerne, can be managed without the input of chemical herbicides (Bellinder et al., 2004; Meiss et. al, 2010). Grass clovers that are cut regularly are known to suppress perennial weed species, such as *Cirsium arvense* (L.) Scop. and *Sonchus arvensis* L. (Melander et al., 2024), and annual weeds are also reduced by periods with grass clovers (Sjursen, 2001; Albrecht, 2005). Melander et al. (2020) showed that including a biennial leguminous green manure crop in an organic crop rotation could reduce the germinable seed bank of annual weeds by 65 % compared to a crop rotation consisting of annual cash crops only. The causes are found in the low input of new weed seeds during the lifetime of well-managed perennial crops and the relatively short longevity of most annual weed seeds (Bohan et al., 2011).

Despite the advantages of herbaceous perennial crops for managing weeds, the adoption of these crops is limited in both conventional and organic arable cropping without livestock. The current lack of revenue from bioenergy and biorefined crops is a major hindrance to farmers refraining from growing cash crops. Plant materials from perennial crops have little use apart from being a valuable forage or green manure. The situation may change with emerging possibilities to use biomass from annual and perennial 'biocrops' for bioenergy, biorefining or other industrial purposes. This may be relevant for both herbaceous crop species and lignocellulosic perennial crops, such as miscanthus and willow. Work is ongoing to improve biorefining technologies, which are important for the production of biobased products including protein for food and feed as well as materials and energy (Møller et al., 2021). Lignocellulosic perennial biocrops may deliver the same benefits as herbaceous perennial biocrops, but the need for chemical herbicides in woody biocrops is uncertain. Depending on species and management, perennial biocrops can be grown for several years without any requirements for chemical herbicides, potentially reducing the use of chemical herbicides substantially at the farm level. However, herbaceous perennial biocrops are not grown in the same way as perennial crops for forages and green manuring, but can have different species mixtures, fertilization regimes, harvest frequencies and crop lifetimes. Crop management for annual biocrops may also differ from when growing these crops for grain. In particular, the biocrop production of green biomasses may be prioritized over the traditional focus on dry seeds rich in starch, protein and oil.

This paper hypothesizes that the herbicide-free cropping of herbaceous perennial biocrops does not result in germinable weed seed banks greater than the cropping of annual biocrops receiving chemical herbicides. Prospectively, the inclusion of periods with herbicide-free cropping of perennial biocrops can result in a lower herbicide consumption at the farm level without deteriorating weed management. Perennial biocrops grown without herbicides will support the goals of integrated pest management (IPM) programmes aiming at reducing reliance on chemical pesticide usage (European Commission, 2020).

The hypothesis was studied in two ongoing long-term cropping system experiments with biocrops at two sites in Denmark. Soil samples were taken at different points along the cropping history with the aim of estimating the development in the sizes of germinable weed seed banks in the different cropping systems. These include systems with both perennial and annual crop species. In addition to estimating germinable seed bank sizes, we were particularly interested in whether herbicide-free perennial cropping systems with biocrops have germinable weed seed banks of similar or lower magnitudes than the annual systems receiving chemical herbicides. One experiment is located on a sandy loam at the Flakkebjerg Research Centre (55.33°N, 11.39°E) and commenced in autumn 2014 while the other started in 2012 on a loamy sand at

Foulumgaard (56º30'N, 09º35'E). At both sites, annual cash crops were grown with chemical herbicides and inversion tillage in the years preceding the experiments. In Flakkebjerg, six annual cropping systems (named ‘$\mathrm{AS_{FL}}$1, 2, 3, 4, 5, 6’) with high chemical herbicide inputs, four partly annual and partly perennial systems (two years with grass ley, named ‘$\mathrm{APS_{FL}}$1, 2, 3, 4’) with medium herbicide inputs and five perennial systems (named ‘miscanthus’, ’grass clover’, ‘red clover’, ‘tall fescue’ and ‘willow’) with medium to no herbicide inputs were selected for soil sampling (Table 1). Table S1 in supporting information lists the crop species grown in the cropping systems with their scientific names. In Foulumgaard, seven annual systems (named ‘$\mathrm{AS_{FO}}$1, 2, 3, 4, 5, 6, 7’) with high inputs of herbicides and two perennial systems (named ‘miscanthus’ and ‘grass clover’) with medium and no herbicide inputs, respectively, were selected (Table 2). The annual systems had different sequences of crops with harvest times that prioritized optimal biomass production rather than grain yield. Mouldboard ploughing followed by seedbed tine cultivation was conducted annually before biocrop establishment in the annual systems. The perennial systems were only primarily tilled in the year of establishing the biocrop. The perennial biocrops were cut regularly at ground level at frequencies and timings that optimized biomass production: usually with several annual cuttings in the herbaceous crops, one annual cutting in miscanthus and one cut every third year in willow. More details on the type of experiment with information on crop establishment, fertilization, harvest dates and management can be found in Larsen et al. (2024).

Chemical herbicides were the primary weed control method in all annual cropping systems supplemented by inter-row hoeing with faba beans and hemp. The perennial systems with grass clover, tall fescue and red clover were grown without any chemical herbicides, except in the establishment year of tall fescue and red clover. Chemical herbicides were used in willow and miscanthus whenever possible and miscanthus was also cultivated between the rows.

Soil samples were collected in mid-October 2014, 2017 and 2021 from all 60 plots (four blocks) at Flakkebjerg and in mid-October 2014 and 2017 from all 36 plots (four repetitions arranged in rows and columns) at Foulumgaard using an auger with a 50-mm diameter. Plot size at Flakkebjerg was 15 x 2.5 m and at Foulumgaard plot lengths were 20 m but with plot widths varying from 3 to 12 m depending on the cropping system. Soil sampling was randomly made within the central plot area 11 x 2 m at Flakkebjerg and 16 x 2 m at Foulumgaard. Ten samples, corresponding to 392.7 cm^3 each, were taken from the plough layer (0-20 cm) in each plot; in total nearly 4 litres of soil was sampled per plot. The ten samples per plot were thoroughly mixed, placed in plastic bags and stored at 4 °C in darkness. Then the samples were placed in polystyrene boxes and set for germination in the glasshouse in late October. Soil sampling, preparing the boxes and the germination procedure followed the description in Scherner et al. (2016). Germination was completed in late October a year later resulting in a germination period of one year. The total number of weed seedlings that emerged per box was obtained by regular counting across the year at the species level. The total numbers per species and weeds in total are presented as the number of germinable seeds m^{-2} in the plough layer that could be obtained within a year.

All the discrete seed bank data were log-transformed and analysed using a generalized linear mixed model with a Poisson distribution of errors (SAS release 9.4, SAS Institute Inc., Cary, N.C.). The fixed effects were the cropping system (treatment) and year including the interaction between the year and cropping system. When more years were included in the analyses, year was also included as a repeated effect with the plot as the subject, assuming an autoregressive correlation structure and variance between years. Random effects were given as a block for the Flakkebjerg data and row and column for the Foulum data to account for the spatial variability (see also Larsen et al., 2024).

The sizes of the germinable weed seed banks in 2014, 2017 and 2021 in the 15 cropping systems in Flakkebjerg are shown in Fig. 1. The initial germinable seed bank size in 2014 did not differ significantly between the plots selected for the cropping systems with the first crops established in 2015; the numbers of germinable seeds were relatively low, mostly < 5,000 seeds m^{-2} (Table S2, supporting information). Miscanthus and willow had developed significantly greater germinable seed banks than all other systems combined in 2017 ($P < 0.0001$). The increase in germinable seed bank sizes was seen in all the most abundant species between the 'miscanthus / willow' group and all other cropping systems in 2017 in Flakkebjerg, notably *Poa annua* L. (P<0.0001), *Chenopodium album* L. (P=0.0006), *Veronica* spp. L. (P<0.0001) and *Lamium purpureum* L. ($P = 0.0040$) (Table S3, supporting information). The germinable seed bank of miscanthus declined from 2017 to 2021 ($P < 0.0001$) while the one for willow almost remained the same. The effects in 2021 in Flakkebjerg were mainly seen in the most abundant species *P. annua*, *C. album*, *Veronica* spp., *Tripleurospermum perforatum* (Mérat) M. Laínz and *L. purpureum* (Table S4, supporting information). The germinable seed bank sizes of grass clover and tall fescue remained relatively constant, while the seed bank of red clover tended to increase from 2017 to 2021 ($P = 0.0572$) (Fig. 1). In 2021, the germinable weed seed banks of grass clover and tall fescue (averaging 3,782 seeds m^{-2} (± 877 S.E.)) were among the smallest of all the systems in Flakkebjerg (Fig. 1). The germinable seed bank of red clover was significantly greater than grass clover and tall fescue combined ($P = 0.0032$) in 2021.

Miscanthus had the greatest germinable weed seed bank in Foulumgaard in both 2014 and 2017 when contrasted with all other cropping systems combined (*P*<0.0001 for both years, Fig. 2). This was particularly reflected in a significant increase of *Lapsana communis* L. (Tables S5 and S6, supporting information). However, the germinable seed bank of miscanthus declined significantly from 2014 to 2017 (*P*=0.0004). Grass clover had a relatively small germinable seed bank size in both years with 6,781 (±1391 S.E.) seeds m^{-2} in 2014 and 4,804 (±1171 S.E.) seeds m^{-2} in 2017. Contrasting grass clover with the annual cropping systems combined did not reveal any significant differences in germinable seed bank sizes in 2014 nor in 2017 (Fig. 2).

The hypothesis stating that the herbicide-free cropping of herbaceous perennial biocrops does not result in germinable weed seed banks greater than those found with annual biocrops receiving chemical herbicides is well supported for grass clover, tall fescue and partly red clover. Grass clover, tall fescue and red clover (2015-2017) developed a dense and competitive crop stand with the ability to regenerate quickly after cutting (personal observations). The lack of tillage and a rapid canopy closure prevented the stimulation of weed seed germination and, consequently, annual weed growth and the input of new weed seeds to the seed bank (Melander et al., 2020). For red clover in Flakkebjerg, however, the crop stand became gradually thinner and less competitive after 2017 (personal observations). This explained the increase in germinable seed bank size in 2021. The management of weeds in miscanthus and willow was more challenging, particularly due to the lack of quickly achieving a dense and competitive canopy following cutting (Feledyn-Szewczyk et al., 2019). The options for direct weed control in miscanthus and willow were limited. These resulted in significant growth of annual weeds until canopy closure eventually happened, particularly in the establishment phase of miscanthus and willow (personal observations). However, the miscanthus germinable seed bank declined from

2017 to 2021 at Flakkebjerg and from 2014 to 2017 at Foulumgaard, suggesting that miscanthus became more competitive once it had established a strong root system that enabled faster regrowth following cutting.

The data on germinable seed bank size in this study reflects the weed problems that have accumulated in the cropping systems during their lifetime and the years ahead of commencing the systems. However, according to Bohan et al. (2011), the majority of weed seeds in arable soils only persist for three years and thus the germinable seed bank sizes found in this study are mostly a result of the impact from the cropping systems and less the legacy from previous years cropping history except for the initial germinable seed bank in Flakkebjerg in 2014. However, the germination method used does not reveal the whole seed bank but only the germinable weed seeds while dormant seeds were not recorded. Scores of the aboveground weed coverage in Flakkebjerg in 2017 and in 2021 (data not shown) and in Foulum in 2017 (data not shown) only correlated clearly with the germinable seed banks of the weediest systems (miscanthus and willow). Data on aboveground weed density or weed coverage were not further prioritized because they just show the weed situation in the biocrop at a specific time point and do not inform robustly about potential future weed problem (Liebman et al, 2021; Shi et al., 2026; Sjursen et al, 2001).

In conclusion, densely growing and frequently cut grass-clover mixtures and tall fescue did not proliferate germinable weed seed banks during 5-7 years of cropping. These biocrops can be managed without any chemical pesticides (except for the establishment year of tall fescue) and hence contribute significantly to the objective of IPM programmes at the farm level depending on the area and duration with biocrops. In contrast, lignocellulosic perennial biocrops, such as willow and miscanthus that are not suited to several cuttings per year and that leave the soil bare for longer periods without effective weed control, may cause significant weed problems in subsequent cash crops.

CRediT author contribution statement

Bo Melander: Data curation, formal analysis, methodology, validation, visualization, writing - original draft, writing - review and editing. **Birte Boelt**: Conceptualization, project administration, planning and managing the experiment in Flakkebjerg, supervision, writing - review and editing. **Poul E, Lærke**: Conceptualization, operating the long-term experiment in Foulum, resources, supervision, writing - review and editing. **Søren U. Larsen**: Conceptualization, operating the long-term experiment in Foulum, resources, supervision, writing - review and editing. **Uffe Jørgen**: Conceptualization, project administration, funding acquisition, writing - review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Funding: This study is supported by the Innovation Fund Denmark Project GrassTools (project number 0224–00091B) and by the Interreg Øresund-Kattegat-Skagerak programme through the project Green Valleys 2.0 (grant number 20358654). Technical assistance from Søren Sommer Petersen, Karen Heinager and Eugene Driessen, Aarhus University, is greatly appreciated.

Appendix A

Supporting information to this article can be found on-line at:

Data availability

Data will be made available on request

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Table 1

Crop sequences in the annual, annual/perennial and perennial cropping systems at Flakkebjerg shown for 2015-2021.

Cropping system	Perenniality / herbicide input***	Year						
		2015	2016	2017	2018	2019	2020	2021
AS$_{FL}$1	Annual/h	Triticale|FW.rape	Maize|W.rye	F.beet	F.beans	Triticale| FW.rape	Maize	F.beet
AS$_{FL}$2	Annual/h	Maize|W.rye	F.beet	Hemp	Trtitcale| FW.rape	Maize|W.rye	F.beet	F.beans
AS$_{FL}$3	Annual/h	F.beet	Hemp	Triticale| FW.rape	Maize|W.rye	F.beet	F.beans|W.ryegr.	Triticale|F.lolium
AS$_{FL}$4	Annual/h	Hemp	Triticale| FW.rape	Maize|W.rye	F.beet	F.beans	Triticale|F.lolium	Maize
AS$_{FL}$5	Annual/h	W.rape|Wheat	Wheat	Wheat|OSR	Barl.|W.rape	W.rape|Wheat	Wheat	W.wheat|OSR
AS$_{FL}$6	Annual/h	Maize	Maize	Maize	Maize	Maize	Maize	Maize
APS$_{FL}$1	Annual_ perennial/m	Barl.|T.fescue	T.fescue	T.fescue	Beet	Barl.|T.fescue	T.fescue	T.fescue
APS$_{FL}$2	Annual_ perennial/m	T.fescue	Barl.	Beet	Barl.|T.fescue	T.fescue	T.fescue	Beet
APS$_{FL}$3	Annual_ perennial/m	T.fescue	Beet	Barl.|T.fescue	T.fescue	T.fescue	Beet	Barl.|T.fescue
APS$_{FL}$4	Annual/ perennial/m	Beet	Barl.|T.fescue	T.fescue	T.fescue	Beet	Barl.|T.fescue	T.fescue
Miscanthus	Perennial/m	Miscanthus	Miscanthus	Miscanthus	Miscanthus	Miscanthus	Miscanthus	Miscanthus
Grass clover	Perennial/a	Grass clover	Grass clover	Grass clover	Grass clover	Grass clover	Grass clover	*Grass clover
Tall fescue	Perennial/l	Barl. + fescue	T.fescue	T.fescue	T.fescue	T.fescue	T.fescue	T.fescue
Red clover	Perennial/l	Barl. + clover	Red clover	Red clover	Red clover	Red clover	Red clover	**Red clover
Willow	Perennial/m	Willow	Willow	Willow	Willow	Willow	Willow	Willow

Vertical lines show the change of crops within the year. Abbreviations: 'Barl.' = spring barley; 'Barl. + fescue' = spring barley undersown with tall fescue; 'Barl. + clover' = spring barley undersown with red clover; 'T.fescue' = tall fescue; 'Wheat' = winter wheat; 'Beet' = sugar beet; 'F.beet' = fodder beet; 'FW.rape' = fodder winter rape; 'F.beans' = faba beans; 'W.rye' = winter rye; 'W.ryegr.' = westerwold ryegrass; 'F.lolium' = festulolium; 'OSR' = oil seed radish.

*The grass clover stand was renewed in 2021 by undersowing red clover in spring barley following inversion tillage.

**The red clover stand was renewed in 2021 by undersowing red clover in spring barley following inversion tillage.

***Chemical herbicide input: h=high, m=medium, l=low, a=absent

Table 2

Crop sequences of the annual (AS_{FO}1-7) and perennial (miscanthus and grass clover) cropping systems shown for 2012-2017 at Foulumgaard.

Cropping system	Perenniallity / herbicide input*	Year					
		2012	2013	2014	2015	2016	2017
AS_{FO}1	Annual/h	Hemp|W.rye	W.rye|Maize	Maize	Maize	Maize	Maize
AS_{FO}2	Annual/h	Oat|Wheat	Triticale	Triticale	Triticale	Triticale	Triticale
AS_{FO}3	Annual/h	Maize	Barl.|W.barley	W.barley|W.rape	W.rape|Wheat	Wheat|OSR	Barl.|W.barley
AS_{FO}4	Annual/h	W.rye	Maize|W.rye	Beet	Hemp|Triticale	Triticale|W.rape	Maize|W.rye
AS_{FO}5	Annual/h	Maize|W.rye	Beet	Hemp|Triticale	Triticale|IR+RC	Maize|W.rye	Beet
AS_{FO}6	Annual/h	Beet	Hemp|Triticale	Triticale|IR+RC	Maize|W.rye	Beet	Hemp|Triticale
AS_{FO}7	Annual/h	Hemp|Triticale	Triticale|W.rye	Maize|W.rye	Beet	Hemp|Triticale	Triticale|W.rape
Miscanthus	Perennial/m	Miscanthus	Miscanthus	Miscanthus	Miscanthus	Miscanthus	Miscanthus
Grass clover	Perennial/a	Grass clover	Grass clover	Grass clover	Grass clover	Grass clover	Grass clover

Vertical lines indicate crop changes within the year. Abbreviations: 'Barl.' = spring barley; 'IR'= Italian ryegrass; 'OSR' = oil seed radish; 'RC' = red clover; 'W.barley' = winter barley; 'W.rape'= winter rape; 'W.rye' = winter rye; 'Wheat' = winter wheat.

*Chemical herbicide input: h=high, m=medium, a=absent

Fig. 1

Germinable seed bank sizes in 2014, 2017 and 2021 assessed for six annual cropping systems (AS_{FL}1, 2, 3, 4, 5, 6), four partly annual / partly perennial systems (APS_{FL}1, 2, 3, 4) and five perennial systems (miscanthus, grass clover, red clover, tall fescue and willow) at Flakkebjerg. The columns show the means of seed numbers m^{-2} with standard error bars.

Fig. 2

Germinable seed bank sizes in 2014 and 2017 assessed for seven annual cropping systems (AS_{FO}1, 2, 3, 4, 5, 6, 7) and two perennial systems (miscanthus and grass clover) at Foulumgaard. The columns show the means of seed numbers m^{-2} with standard error bars.

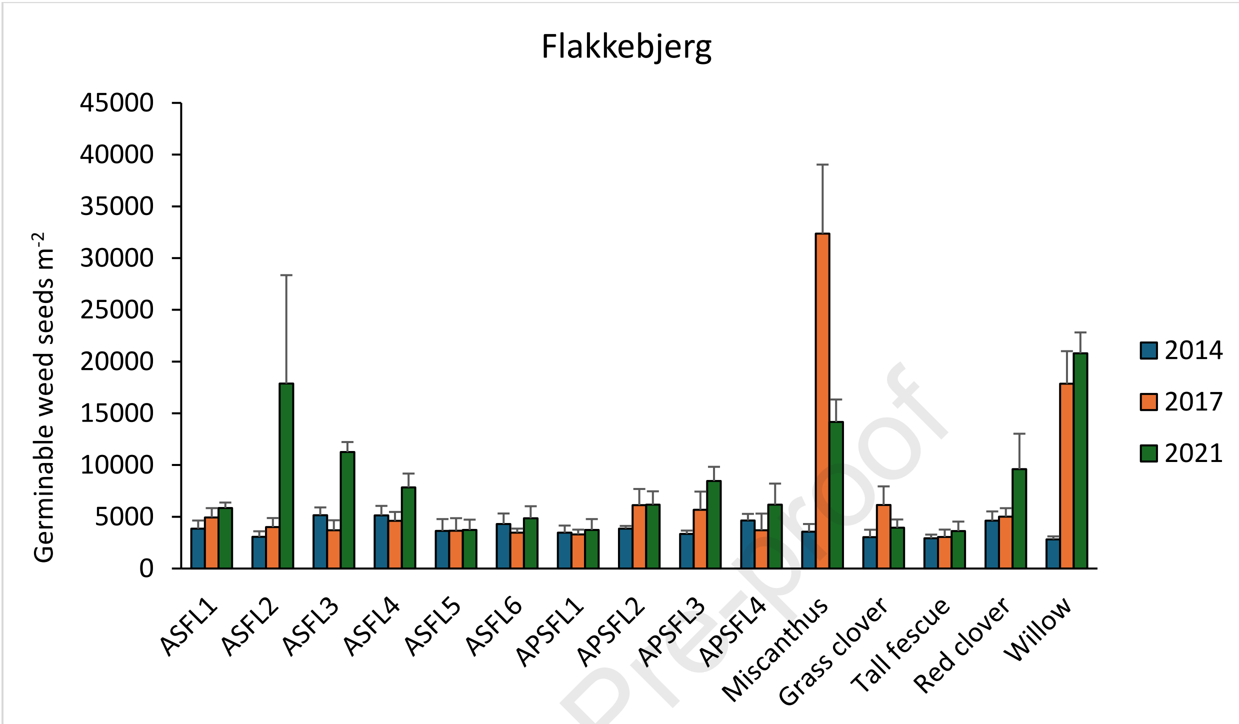
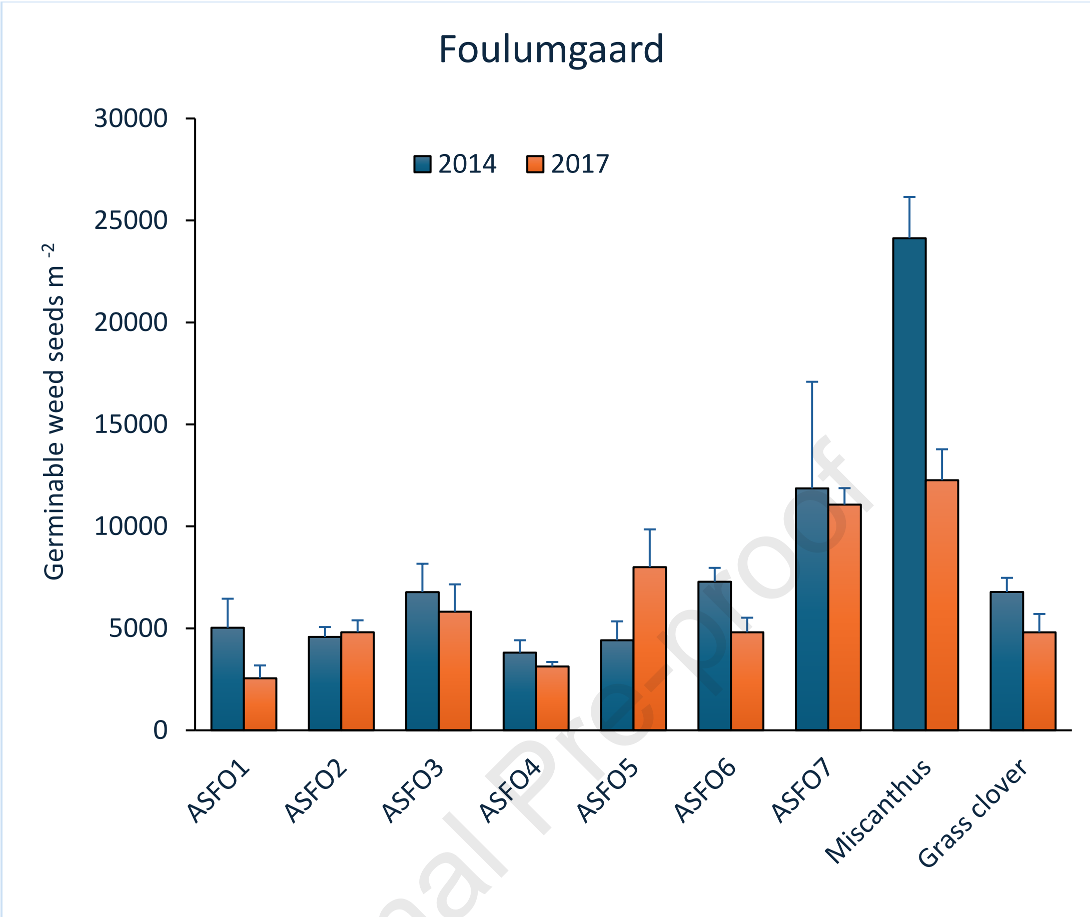

Flakkebjerg
Germinable weed seeds m-2
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
ASFL1
ASFL2
ASFL3
ASFL4
ASFL5
ASFL6
APSFL1
APSFL2
APSFL3
APSFL4
Miscanthus
Grass clover
Tall fescue
Red clover
Willow
2014
2017
2021

Fig. 1



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359 **Fig. 2**

Highlights

- Perennial herbaceous biocrops can reduce inputs of chemical pesticides
- Lignocellulosic perennial biocrops may result in significant future weed problems
- Some biocrops can benefit integrated pest management programs (IPM) at the farm level

Conflicts of Interest

Authors Bo Melander, Birte Boelt, Poul E. Lærke, Søren U. Larsen and Uffe Jørgensen declare no conflicts of interest.

Bo Melander