FACULTY OF LIFE SCIENCES
UNIVERSITY OF COPENHAGEN

Phenology and source-sink dynamics of carbohydrates in relation to management of perennial weeds: *Cirsium arvense* (L.) Scop and *Tussilago farfara* L.

PhD thesis

Libère Nkurunziza

Academic advisor: Jens Carl Streibig, Professor

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Department of Agriculture and Ecology, Crop Sciences
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Preface

This thesis was submitted as partial fulfillment for the degree of Doctor of Philosophy (PhD) requirements. The work was done at the Department of Agriculture and Ecology, at the University of Copenhagen, under supervision of Professor Jens Carl Streibig. In appendices of the present thesis, four manuscripts produced during the PhD education period are given.

This work has involved the help of many people without whom it would not have been what it is. First and foremost I am deeply indebted to Jens Carl Streibig. Throughout the doctoral studies, he has been my principal supervisor and he has never ceased to be preoccupied by my progress as an employee or a student. His attitude toward scientific research and educational goals has taught me a lot and inspired me.

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Libère Nkurunziza, Taastrup, June 2010

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Summary

Perennial weed infestations are putting severe constraints on organic and conventional farming. *Cirsium arvense* (L.) Scop. and *Tussilago farfara* L. have high vegetative regeneration capacity from underground organs. These organs can reach deep soil layers, which prevent them from being easily uprooted. In our research, we primarily addressed the importance of the source-sink dynamics of carbohydrates at different phenological stages and related results to eventual management strategies.

Experimental work was carried out under greenhouse and/or growth chamber conditions. The first study spanned from roots/rhizomes planting to shoot establishment. The second study began after establishment and included the rosette to the flowering stages. The third study was about the storage of carbohydrates in the underground parts. And finally, a fourth study aimed at unraveling the effect of drought on new shoots of these perennials.

To determine the depletion of carbohydrate storage associated with shoot development, we destructively sampled roots of *C. arvense* and rhizomes of *T. farfara* from which nonstructural carbohydrates were measured using HPLC. We found that fructan was highest at the planting time, but it decreased by releasing fructose as shooting growth progressed. However, before the released fructose was depleted, carbohydrates were reloaded by photo-assimilates. This was demonstrated by ^{14}C labelling to track the commencement of the basipetal translocation of photo-assimilates. The conclusion was that appreciable basipetal translocation starts at 6 and 8 fully developed leaves for *T. farfara* and *C. arvense*, respectively. This falls between 500 and 600 degree days or around 21 to 23 days after emergence under our experimental conditions.

After shoot establishment we measured the maximum net photosynthesis of disturbed and undisturbed clones over a period of 3 weeks, from the rosette stage to the flowering stage. Maximum net photosynthesis decreased over time, but there were no differences between disturbed and undisturbed clones. Our conclusion was that the physiological integration found in other clonal species seems to be absent in *C. arvense* and *T. farfara*, suggesting that shoots are autonomous.

To understand carbohydrate storage, juvenile and mature plants were grown at different temperatures. The results showed that the polymerization of fructans was

associated with low temperatures for *C. arvense* but not for *T. farfara*. Polymerization of fructans in roots/rhizomes was not significantly different between juvenile and mature plants. Only the dry weight of shoots from juvenile plants reflected the differences found in carbohydrate content. The conclusion is that the continuous growth of underground propagules facilitates the survival of these two perennials and thus complicates their control.

Gradient soil water content during the establishment period of shoots of *C. arvense*, *T. farfara* and *Elytrigia repens* showed that *E. repens* is more tolerant to water stress than broadleaf *T. farfara* and *C. arvense*. *T. farfara* is more prone to water stress compared to *C. arvense* if we consider the relationship between the soil water content and shoot biomass. This susceptibility might give an opportunity window for managing broadleaf perennial weeds.

Sammendrag

Flerårige ukrudt medfører alvorlige begrænsninger for planteproduktionen i økologisk og konventionelt landbrug. *Cirsium arvense* (L.) Scop og *Tussilago farfara* L. har høj vegetative regenereringsevne fra de underjordiske organer. Disse organer kan nå dybe jordlag, der forhindrer dem i at blive trukket op og udtørret på jordoverfladen. I vores forskning undersøgte vi primært betydningen af ”source-sink” dynamik for kulhydrater i forskellige fænologiske faser og relaterede resultaterne til mulige bekæmpelsesstrategier. Det eksperimentelle arbejde blev udført i drivhus, og / eller i vækstkammer.

Den første undersøgelse spændte fra plantning af rødder/rhizomer til skudetablering. Den anden undersøgelse blev indledt efter etableringen af rosetstadiet og indtil blomstring. Den tredje undersøgelse drejede sig om oplagring af kulhydrater i de underjordiske dele, og den fjerde undersøgelse studerede virkningen af tørke på nye skud af disse flerårige arter.

For at bestemme nedbrydningen af kulhydrat i forbindelse med dannelse af nye skud, brugte vi en destruktiv prøveudtagning af rødder fra *C. arvense* og jordstængler fra *T. farfara* og målte opløselige kulhydrater ved hjælp af HPLC. Fruktaner var højest ved plantningen, men faldt ved at frigive fruktose under skuddannelsen. Men før den frigivne fruktose var udtømt, begyndte oplagringen igen af fotosyntesens kulhydrater. Dette blev påvist med 14C mærkning. Konklusionen var, at basipetal transport begynder ved 6 og 8 fuldt udviklet blade for henholdsvis T. farfara og C. arvense. Dette falder mellem 500 og 600 graddage eller omkring 21 til 23 dage efter ny skuddannelse.

Efter etablering målte vi den maksimale nettofotosyntese af forstyrret og uforstyrret kloner over en periode på 3 uger, fra roset- til blomstringsstadiet. Fotosyntesens nettoeffekt faldt over tid, men der var ingen forskel mellem forstyrret og uforstyrret kloner. Med andre ord den fysiologiske integration som findes i andre flerårige arter med underjordiske vegetative organer synes at være fraværende i *C. arvense* og *T. farfara*, og det tyder på, at skuddene er autonome.

Unge og ældre vegetative planter dyrket ved forskellige temperaturer viste, at størrelsen af fruktanreserven var forbundet med lave temperaturer for *C. arvense*, men ikke for *T. farfara*. Størrelserne af fruktankoncentrationen i rødder/rhizomer var ikke signifikant forskellig mellem unge og ældre planter. Kun tørvægten af unge planter

afspejlede forskelle i kulhydratindholdet. Konklusionen er, at den fortsatte vækst af rødder og/eller rhizomer letter overlevelse af disse to flerårige arter og dermed komplicerer deres bekæmpelse.

Gradienter i jordens vandindhold under etableringsperioden af *C. arvense*, *T. farfara* og *Elytrigia repens* viste, at *E. repens* er mere tolerant overfor vandstress end de bredbladede *T. farfara* og *C. arvense*. *T. farfara* er mere tilbøjelige til at lide af vandstress i forhold til *C. arvense*, hvis vi ser på forholdet mellem jordens vandindhold og biomasseproduktion. Denne følsomhed kan give en mulighed for at rammer ukrudtet når det er mest tørkestresset.

Résumé

Les adventices pérennes posent des sérieux problèmes à l'agriculture biologique des pays Nordiques mais aussi des autres régions. Parmi les espèces les plus rencontrées, nous reconnaissons *Cirsium arvense* (L.) Scop and *Tussilago farfara* L. Ces deux espèces sont connues pour leur capacité de régénération végétative élevée émanant de leur organs souterrains, racines et rhizomes, qui peuvent pénétrer jusqu'à plus d'un mètre de profondeur. Au cours de notre travail, nôtre attention s'est focalisé sur la compréhension des allocations des hydrates de carbone au cours de la phénologie des deux espèces afin de contribuer à l'amélioration des méthodes de contrôle.

Pour ce faire, nous avons échelonné notre expérimentation sur trois principales phases phénologiques avec quatre études. La première phase expérimentale avait comme objectif de suivre la dégradation des réserves associée à l'émergence des pousses ainsi que la translocation des photo-assimilâts vers les parties souterraines. Cette phase commençait avec la date de la plantation des fragments de racines pour *C. arvense* et de rhizomes pour *T. farfara* jusqu'à peu prêt quatre semaines après l'émergence des pousses. L'étude de la seconde phase examinait si le raccordement des pousses d'un clone a un effet sur pouvoir photosynthétique. L'expérimentation était faite à partir de 5 semaines après l'émergence de pousses pour se terminer à la floraison. La troisième étude concernait tout le cycle végétatif et l'importance était centrée sur le stockage des hydrates de carbones à des températures et âges variés. Enfin, nous nous sommes intéressés aussi sur l'impact que le déficit en eau du sol pourrait occasionner pendant la période d'émergence des pousses.

Afin de déterminer la dégradation des réserves des hydrates de carbone dans les racines et rhizomes au cours de l'émergence des pousses, le saccharose, le glucose, le fructose, l'amidon et les fructanes des racines et rhizomes reprises quelques temps après la plantation étaient mesurés. Les fructanes se trouvent être les plus importantes réserves et leur dégradation résulte en une augmentation du fructose. Néanmoins, avant que le fructose ne soit complètement utilisé, le transport des photo-assimilâts récents vers les racines et les rhizomes était déjà constaté. Ceci était montré par la technique d'isotope carbone 14 utilisé pour suivre la translocation des photo-assimilâts. De nouvelles réserves sont alors de nouveau constituées donnant ainsi la possibilité d'émergence des nouvelles

pousses pour constituer un clone. A 6 feuilles pour *T. farfara* et 8 feuilles pour *C. arvense*, soit 500 à 600 degrés-jours, la reconstitution de l'énergie dans les rhizomes et racines était déjà constaté.

Le fait de couper une ou plusieurs pousses dans un clone n'a pas d'effet sur les autres pousses restées intactes. Ceci a été démontré par une étude sur la photosynthèse mesurée sur les clones sur lesquelles soit un certain nombre de pousses est coupé ou alors laissé complètement intacts. Ces mesures faites sur trois semaines ont démontré que chacune des pousses d'une clone de *C. arvense* et *T. farfara* est autonome contrairement à ce qui est connu chez d'autres espèces.

La température a affecté le stockage des fructanes pour le cas de *C. arvense* mais pas *T. farfara*. Les basses températures favorisent le métabolisme des longues chaînes des fructanes. Quatre semaines après l'émergence, si les pousses sont exposées à des basses températures, la longueur des chaînes des fructanes est la même que celle des plants majeures. La capacité générative des fragments de racines et rhizomes replantés, après des températures variées, ont montré que les biomasses des pousses obtenues des racines ou rhizomes des jeunes plantes diffèrent. Ceci se refléte aussi dans le rythme d'émergence des pousses. En conclusion, les racines et rhizomes connaissent une croissance continue et accumule des réserves tout au long de la saison végétative. Ceci constitue pour les deux espèces étudiées un bon moyen de survie et constitue une difficulté quant aux possibilités de les contrôler.

L'étude sur l'effet de la teneur en eau du sol pendant l'émergence et la croissance des pousses de *C. arvense*, *T. farfara* and *Elytrigia repens* aura montré qu'il pourrait y avoir une marge de manœuvre quant aux stratégies utilisées pour contrôler les mauvaises herbes vivaces avec des feuilles larges, dicotylédones. *E. repens* était la seule espèce à pouvoir tolérer les faibles teneurs en eau dans le sol. *T. farfara* est plus susceptible à une faible teneur en eau du sol que *C. arvense*.

List of papers

I. **Nkurunziza L. & Streibig JC.** Carbohydrate dynamics during early growth of *Cirsium arvense* and *Tussilago farfara*. Submitted to *Weed Research* on 29th, January 2010

II. **Nkurunziza L., Rosenqvist E. & Streibig JC.** Photosynthesis and growth of newly established shoots of *Cirsium arvense* (L.) Scop. and *Tussilago farfara* L. are resource independent. Accepted for publication. *Weed Research*

III. **Nkurunziza L. & Streibig JC**. Temperature and age effects on carbohydrate storage and regeneration of of *Cirsium arvense* (L.) Scop and *Tussilago farfara* (L.). To be submitted to *Weed Science*

IV. **Nkurunziza L., Andreasen C., Liu F. & Streibig JC.** Drought tolerance and management of perennial weeds. To be submitted to *Weed Science*

1. Introduction

1.1. Background

The seasons are astronomical, meteorological, biological, and agricultural (Battey 2000). Seasonal and climatic changes are some of the non-living or *abiotic* components of the environment that influence the living or *biotic* components. Seasonal changes can include variations in day length, temperature, precipitation, etc. Biologically, research on seasonality and phenology has received substantial attention (Lieth 1974) because the success of an ecosystem or a food chain depends on the timing of phenological events.

Phenology, the timing of life cycle events in plants, has been used as a predictive strategy for weed and crop competition (Huang *et al.* 2001) but most predictive models in weed science deal with annual weed species, thus seed to seed development. Perennial weeds that involve asexual reproduction present interesting aspects that need more investigation. As with seeds, vegetative buds undergo unfavorable conditions as resistant structures to abiotic and biotic stresses (water deficit, extreme temperatures, salinity, herbivores, pests, etc.) in a dormant state that is an important characteristic of perenniality (Kamenetsky 2009; Rohde & Bhalerao 2007). For example, vegetative bud tissues can survive - 40 C or below (Jones 1992). Even though the dormancy or the capacity of vegetative propagules to survive stress conditions are not fully understood (Anderson & Choa 2001; Borchert 1991, Klimesova & Klimes 2006), it can be considered as a stepping-stone for species survival and spread. Horvath (2009) described how flowering and dormancy of perennials are regulated by common mechanisms.

Carbohydrates form the main energy storage in regenerative propagules and they act not only as a fuel for growth but also as sensing and signaling compounds (Gibson 2004; Gibson 2005; Gupta & Kaur 2005; Lei *et al.* 2007; Rolland *et al.* 2000; Rolland *et al.* 2002; Rolland & Sheen 2005; Rolland *et al.* 2006; Rook *et al.* 1998; Sheen *et al.* 2007) in synergy with other metabolites. The alternations of carbohydrate metabolism and allocation associated with phenological events have been demonstrated in several perennial species in conjunction with environmental cues and phenological time (Anderson *et al.* 2005; Asano *et al.* 2006; Bansal & Germino 2009; Becker & Fawcett

1998; Cyr *et al.* 1990; Eshghi *et al.* 2007; Gesch *et al.* 2007; Horvath 2009; Wilson *et al.* 2001; Wilson *et al.* 2006). It stems from these studies that the sources and sinks are interesting in order to harness the management of perennial weeds. So far, sources and sinks of carbohydrates have been described more fully in woody plants (Kozlowski 1992; Bansal & Germino 2009; Shepherd 1985) than in herbaceous perennials despite the fact that the latter constitute a plant group with great economical importance in agro-ecosystems and rangelands.

Bearing in mind the importance of carbohydrates as mediating compounds in endogenous rhythms responsible for phenology, we think that their quantification in response to environmental stimuli could give added value to the knowledge on the biology and the ecology of perennial weeds. For example, the understanding of the carbohydrate source-sink relations associated with the phenology of perennial weeds will allow researchers and farmers to know when the weeds are most susceptible to control strategies.

The use of the source-sink relations might be the current need for control of some species like creeping thistle (*Cirsium arvense* (L.) Scop.) and coltsfoot (*Tussilago farfara* L.), which are considered as big challenges for both conventional and organic farming. Research on *C. arvense* has been conducted for more than one hundred years but its effective control has not yet been reached. In the same manner, *T. farfara* has been known in agriculture as a weed for over than 50 years (Bakker 1960). The use of herbicides has a relatively large impact on lowering infestation by these perennial weeds. However, the concern associated with side-effects of herbicides on environmental and human health has been increasing the last few decades and has led to a search for new farming strategies. The introduction of organic farming and the restrictions on herbicides has led to an increase of perennial weeds (Andreasen & Stryhn 2008; Thomas *et al.* 2004; Zanin *et al.* 1997). These changes in farming systems require new and efficient methods to manage noxious perennial weeds like *C. arvense* and *T. farfara*.

1.2. Problem statement and research questions

Perennial weeds, mostly *Elymus repens* (L.) Gould, *C. arvense*, *Rumex crispus* L., and *T. farfara* are putting severe constraints on organic production in Denmark and other

temperate regions (Andreasen & Stryhn 2008; Rydberg & Milberg 2000; Salonen & Hyvonen 2002). Their high reproduction and dispersal capacity resulting from the fragmentation of their vegetative propagules by soil tillage are their major sources of success.

Direct and indirect control methods of perennial weeds are not satisfactory in organic farming. The attempts to improve both direct and cultural control strategies (Graglia *et al*. 2006; Koch & Rademacher 1966; Lauringson *et al.* 2001; Seibutis & Feiza 2008) remained unsatisfactory because organic growers could not accept high treatment intensities. Among cultural control methods, perennials are traditionally controlled by repeated and prolonged stubble cultivation in late summer and autumn. But this method conflicts with one of the objectives of organic farming; i.e., to retain nutrients in the upper soil layer by keeping the soil covered with plants during the autumn and winter. Fewer and timelier treatments therefore are required for efficient control of perennials.

In addition to the reluctance of intense interventions by growers and the violation of organic farming objectives, *C. arvense* and *T. farfara* have a special biology that renders them even more difficult to control. For example, *C. arvense* remains a problematic weed under conventional and organic farming because of its selective pressure preserved by its dual regenerative capacity and high genetic variation as increased by dioecy in sexual reproduction (Hettwer & Gerowitt 2004; Slotta *et al.* 2006; Tiley 2010). Another important characteristic of this weed is its deep root system (Reintam *et al.* 2008; Niederstrasser & Gerowitt 2008). This attribute is also found in *T. farfara* and renders them ill-suited to organic farming control strategies. We think that knowledge of sources and sinks of carbohydrate dynamics can improve perennial weed management strategies by reducing infestations arising from asexual regeneration.

The overall objective of this work was to relate sources and sinks of carbohydrates to phenological events and elaborate on eventual control strategies for *C. arvense* and *T. farfara*. We emphasized two main periods in their life cycle when they might be more vulnerable because they have either little or no photosynthetic activity to assist them to recover: before and after dormancy. Management of perennial weeds before dormancy would correspond to disturbance of carbohydrate storage whereas their management after dormancy would mean disturbance of the early growth that uses

carbohydrate reserves. Water is also regarded as a major player in the phenological changes and carbohydrate allocations (Jones 1992; Lundmark *et al.* 2006; Tworkoski 1992). Therefore, in one study, we aimed at investigating the effects of water stress on the early growth of the two perennial weeds in comparison with *E. repens*

In the three first studies (papers I, II and III), *C. arvense* and *T. farfara* were used. In the fourth study (paper IV), *E. repens* was included for comparison. The first study spanned the planting of roots/rhizomes to shoot establishment. The second study started after weed establishment, and included the rosette and the flowering stages. The third study dealt with the accumulation of carbohydrates in the underground parts for both juvenile and mature plants. The last study addressed again the period of sprouting and establishment under soil water deficit.

The following four research questions were addressed:

1. Can we use carbohydrate dynamics to predict when the storage is depleted and when the photo-assimilates start the basipetal translocation during the early growth? ,

> **Hypothesis**: Photo-assimilates start the basipetal translocation after carbohydrate storage in underground organs is depleted.

2. Does the interconnectedness of newly established shoots present benefits for perennial weeds in terms of competitiveness and fitness?

> **Hypothesis**: Net photosynthesis of shoots connected via roots/rhizomes is affected by cutting adjacent interconnected shoots.

3. To what extent does carbohydrate storage differ at different temperature conditions and various developmental stages?

> **Hypothesis**: Less carbohydrate storage is found in juvenile clones and at high temperatures.

4. Can soil water management affect the sprouting and establishment of perennial weeds?

> **Hypothesis**: Deeply rooted and broadleaf perennial weeds, *C. arvense* and *T. farfara*, are more susceptible to water stress than shallowly rooted *E. repens*.

2. Literature review

2.1. Arable land perennial weeds: Biology and ecology

2.1.1. Herbaceous perennial weeds

Taxonomically, perennial weeds are distinguished from annual weeds in that they continue their lifecycle for three or more years. Further classifications put individual species into terrestrial or aquatic perennials, woody or herbaceous, simple herbaceous or creeping herbaceous perennials. Herbaceous perennials are the most troublesome weeds in agriculture, and their biology and ecology have been compared for selected species (Anderson 1999; Håkansson 2003a; Håkansson 2003c; Zimdahl 1993). In Table 1, we summarized characteristics of selected important species of herbaceous perennial weeds. It appears that most perennial species have both sexual and asexual reproduction means. The types and location of reproductive buds, which are responsible for asexual reproduction, differentiate them. According to Anderson (1999), herbaceous perennials have succulent, non-woody, aboveground vegetation that is killed by severe drought, frost, and freezing temperatures. Stems of herbaceous perennials generally undergo little or no secondary growth. In this part of the literature review, we only emphasize the biology and the ecology of two very deep-rooted creeping perennial weeds: *C. arvense* and *T. farfara*.

2.1.2. *Cirsium arvense* and *Tussilago farfara*

C. arvense has been more researched than *T. farfara*. In addition to individual experimental studies on *C. arvense*, several reviews on it have been published, and the most extensive ones are Donald (1994) and Tiley (2010). *T. farfara* has not yet received that much attention even though it has always been an important weed (Bakker 1960, Myerscough & Whitehead 1966, Myerscough & Whitehead 1967). The most recent publication addressing the biology and ecology of *T. farfara* is from Pfeiffer (2008). Other recent studies exploring the flora of northern countries revealed that *T. farfara*, along with other perennial weeds, is on the list of troublesome weeds in organic farming (Andreasen & Stryhn 2008; Rydberg & Milberg 2000; Salonen & Hyvonen 2002).

Table 1: Characteristics of selected herbaceous perennial weeds in cultivated crops

Group: location of reproductive buds (Bud bearing overwintering organs)	Species	Depth of vegetative reproductive parts	Importance of reproduction by seeds
Grass: Creeping rhizomes	*Sorghum halepense*	Shallow	Very
	Elytrigia repens	Shallow	Moderately
	Poa spp.	Aboveground	
Grass: Creeping rhizomes and stolons	*Cynodon dactylon*	Shallow	Very sparse
	Agrostis spp.	Shallow and aboveground	Very sparse
Grasslike: creeping tubers	*Cyperus rotondus*	Shallow	Little
	Cyperus esculentus	Shallow	Little
Noncreeping bulbs	*Allium spp.*	Shallow	No seed
Simple perennial (broadleaved): taproot and/or root crowns	*Taraxacum spp.*	Shallow	Important
	Rumex crispus	Very shallow	Very
	Plantago spp.	-	
Creeping perennial (broadleaved): Horizontal roots	*Cirsium arvense*	Very deep	Important
	Asclepias syriaca	Very deep	Important
	Convolvulus arvensis	Deep	Important
	Apocynum cannabinum	-	Important
	Cardaria spp.	Deep	Important
	Solanum spp.	Deep	Important
	Euphorbia esula	Deep	Very
	Rumex acetocella	Very shallow	Very
	Sonchus arvensis	Very deep	Important
Creeping perennial (broadleaved): horizontal rhizomes	*Urtica dioica*	Very shallow	Very
	Vernonia baldwinii	-	Very
	Tussilgo farfara	Very deep	Important
	Calystegia sepium	Deep	Rarely
	Pteridium aquilinum	Deep	No seed
Other perennial broadleaved: Aerial runners, stolons or creeping stems	*Oxalis spp.*	Shallow	Important in some
	Achillea millefolium	Very shallow	Very
	Equisetum arvense	Deep	No seed
	Veronica filiformis	Aboveground	No seed

Very shallow: 15-25; Shallow: 26-45 cm; Deep: down to 1m, Very deep: Greater than 3m

Source: Anderson (1999) and Zimdhal (1993)

C. arvense and *T. farfara*, both in the *Asteraceae*, are creeping types of herbaceous weeds (see Table 1), and they have several characteristics in common. In the 1960s, Bakker (1960) published a comparative study on the two species due to their rapid establishment and spread that took place from 1947 in newly reclaimed polders of the Zuiderzee, the Netherlands. Findings summarized in this comparative study were similar to those of Korsmo (1954). From a biological point of view, *C. arvense* and *T. farfara* reproduce sexually and vegetatively.

Seed dissemination is mainly facilitated by wind and seeds can travel 2 km and 4 km for *C. arvense* and *T. farfara*, respectively. The viability of seeds is higher in *C. arvense* (up to 30 months) than *T. farfara* (4 months), and seed dormancy is not proven yet in either of the species. Temperature, depth at which the seeds are found, water content and aeration of the soil have been other factors reported to influence the rate of emergence in both *C. arvense* and *T. farfara* (Bakker, 1960). The morphology of seedlings is described in Korsmo (1954). Seedlings are characterized by a tap root for the first 6 weeks after which horizontal roots/rhizomes arise from the tap root.

Before seedlings acquire the ability to propagate vegetatively, they are susceptible to adverse environmental conditions and agricultural practices that cause mortality (Bakker, 1960). Soil water content, soil aeration and light intensity influence seedling growth. *C. arvense* form an erect plant (Fig 1b) whereas *T. farfara* has a compacted stem (Fig 1a). The flowering in *C. arvense* occurs during late vegetative growth whilst for *T. farfara* it takes place early in the vegetative growth at the start of the second year after seedling emergence. Seed outputs are in the range of 3,500 – 40,000 and 1,000 – 8,000 per plant for *C. arvense* and *T. farfara*, respectively (Bakker, 1960).

Vegetative propagules of *C. arvense* are horizontal roots bearing adventitious buds, whereas *T. farfara* has horizontal rhizomes with axillary buds covered by scaly leaves, which serve the purpose of regeneration. Established from seeds, the primary root of *T. farfara* dies three to four months after germination. The radial extension of the seedlings of both species can be as much as 0.75 – 1.25 m in the first year (Bakker, 1960). In the absence of control, it develops into an extensive, spreading root/rhizome system that becomes the major reproduction means during the subsequent years. Two sources of propagation are then observed. The functioning of the seed and bud banks is represented in Fig 2. Roots of *C. arvense* have been traced to a depth of 6 meters, and small pieces of root have produced 20 meters of new roots in 2 years (Persons & Cuthbertson 2001). After only one year a small piece of root of *C. arvense* can produce plants covering 25 m^2 and it is not uncommon to see patches with 130 shoots per m^2 (Persons & Cuthbertson 2001). Within 40 days after emergence, a 5 cm long rhizome of *T. farfara* was able to produce many and thickened horizontal rhizomes (Fig. 1a). Fig 1 c & d illustrate the shoot development from horizontal roots or rhizomes.

C. arvense originated from Euro-Asia and it is geographically distributed in Europe, Asia, North America, Australia and North Africa (Tiley 2010). Its habitat is the subhumid cool-temperate regions where it occurs in open, moderately warm situations up to subalpine levels, usually on the more nitrogenous, deep loam soils (Persons & Cuthbertson 2001). *T. farfara* is distributed throughout the whole of Europe, to beyond latitude 70^{o} N., North America, Central and Northern Asia (Korsmo 1956) on various types of soils.

Among numerous reasons why *C. arvense* remains a troublesome weed, the most striking reasons given in a recent review are:

i) "*the success and persistence derives from an extensive, far-creeping and deep rooting system which ensures survival and rapid vegetative spread under a wide range of soil and management conditions, and a means of escape from sub-aerial control treatments. New adventitious buds capable of shoot development can arise at any point along horizontal roots, even when these are cut into pieces or damaged. Roots buds remain dormant until released from dormancy through damage and decay of the aerial shoots. Carbohydrate root reserves, stored in swollen cortical tissue, fall to a minimum just before flowering and are then replenished for perennation during the subsequent winter. Strategies for control aim to treat the plant when the root carbohydrates are at the minimum, to exhaust these reserves and to prevent replenishment for further perennation"* (Tiley 2010)

ii) *"A combination of dioecy and vegetative reproduction has resulted in the maintenance of genetic diversities within populations allowing efficient colonization and persistence, contributing greatly to success in the species."* (Tiley 2010)

These two conclusions have also been reached in other findings on *C. arvense* (Hettwer & Gerowitt 2004, Slotta *et al*. 2006, Reintam *et al.* 2008, Niederstrasser & Gerowitt 2008).

In the following chapters, our attention will be focused mainly on phenological changes based on vegetative reproduction from bud banks rather than seeds.

(a)

(b)

(c)

(d)

Figure 1: Selected illustrations of *C. arvense* and *T. farfara*. (a) Picture showing the amount of thickened rhizomes developed 40 days after emergence of *T. farfara* from a rhizome fragment of 5 cm. (b) Picture of *C. arvense* stem with an inflorescence. (c) Illustration showing the root system of *C. arvense* with new shoots from the horizontal roots. (d) Illustration of rhizomes of *T. farfara*, inflorescence from the previous year flowers and new shoots developed from horizontal rhizomes. (c) & (d) are adapted from Korsmo *et al*. (1981).

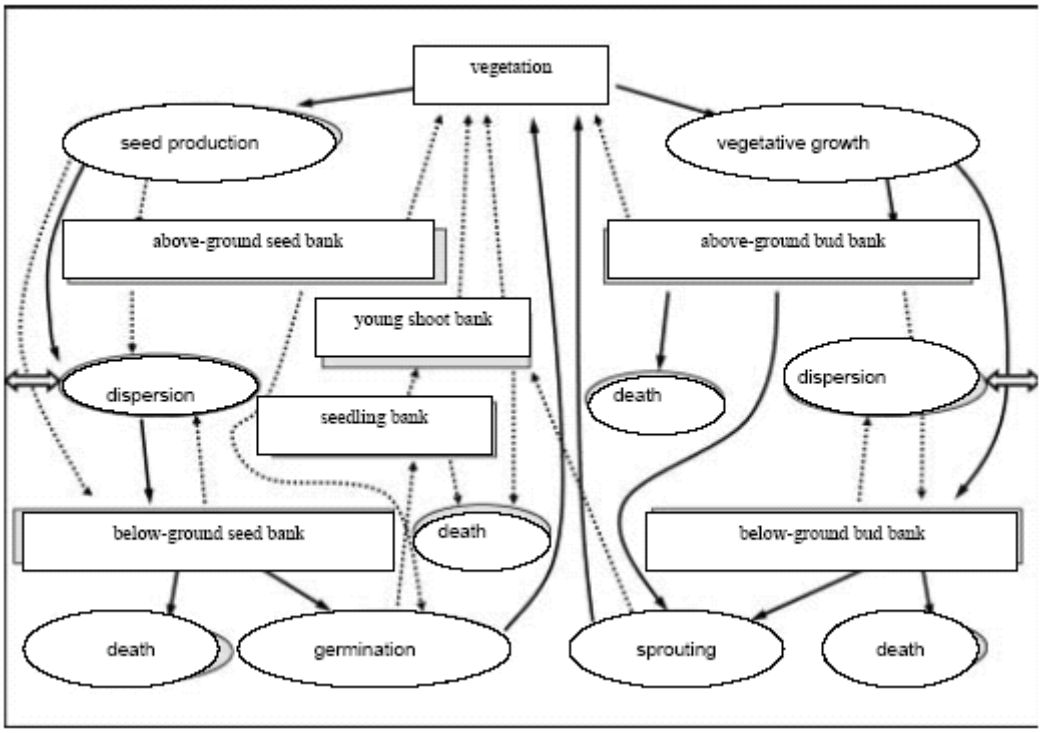

Figure 2: Functioning of the seed and bud banks. Boxes denote emerged vegetation and stored propagules, ellipses indicate processes. Solid arrows describe the most frequent pathways; dotted arrows less frequent pathways. Box arrows directed into and out of the system are inputs (import of diaspores) and outputs (export of diaspores). Adapted from Simpson *et al* (1989)

2.2. Phenology and carbohydrate dynamics

2. 2.1. From sprouting to the compensation point

Sprouting forms and their driving forces in clonal plants have been studied for vascular plants in general (Groff & Kaplan 1988), stoloniferous and rhizomatous plants (Sachs 2001) and root-sprouters (Klimesova & Martinkova 2004). Despite the differences in terminology, the information obtained from these studies has shown three types of sprouting: additive, necessary and regenerative sprouting. For example, using the sprouting from roots (Klimesova & Martinkova 2004), additive sprouting means that root-shoots arise during normal ontogeny but they are not necessary to complete the plant's life cycle. For necessary sprouting, root-shoots are necessary for flowering or over-wintering of the plant. Finally, for regenerative sprouting, root-shoots arise only after injury to a plant. Sprouting factors are known to be both external and internal to the plant. External factors include the cessation of abiotic stress (e.g., water deficit, extreme

temperature and photoperiod, inadequate nutrient level) and disturbances (e.g., removal or injury) of the aboveground plant parts. Internal factors triggering the sprouting are, for example, the plant age, life-history mode and stage.

Morphological changes accompanying dormancy and its break have not been elucidated for herbaceous perennials, but referring to research on trees, vegetative bud sprouting after dormancy break is a result of the release of cell to cell communication via plasmodesmata (Rinne *et al.* 2001). This enables remobilization of water and carbohydrate reserves. This is comparable to the water imbibition of seed for the embryonic axis to elongate and remobilize stored reserves during germination (Bewley *et al.* 2000).

Table 2 shows the importance of carbohydrates in major storage organs of some important seed crops. These carbohydrate storages are involved in early growth. In the same way, the importance of nonstructural carbohydrates is highlighted in perennial weeds (Fig. 3), and their depletion coincide with the period of sprouting and early growth (Fig. 4). In spite of continuous sprouting of new shoots in herbaceous perennials, the figures show also the source and sink of carbohydrates. Carbohydrates serve as a source of energy during the early growth before the plant can produce its own energy through photosynthesis. From that time, they are also involved in multiple and functional roles in mediating a wide range of plant growth and environmental responses (Rolland *et al.* 2002; Sheen *et al.* 2007). For example, they have a role in the resumption of protein synthesis in early growth (Bewley *et al.* 2000) and they also give rise to enzymes and hormones involved in the further metabolic signaling for plant growth (Halford & Paul 2003).

From a weed management perspective, before photo-assimilates exceed carbohydrate amounts used for respiration and growth of shoots (time referred to as compensation point), shoots only depend on stored carbohydrates. Compensation point is a pivotal time because it determines the start of increased weed-crop competition. A few studies on the determination of the compensation point have been conducted (Gustavsson 1997; Håkansson 2003b; Kvist & Håkansson 1985), but the genetic variation of species, populations and environmental conditions dictate more studies and new methods of evaluation.

Table 2: Storage reserves of some important crop species

	Average percent composition			
	Protein	Oil	Carbohydrate	Major storage organs
Cereals				
Barley	12	3	76	Endosperm
Maize	10	5	80	Endosperm
Oats	13	8	66	Endosperm
Wheat	12	2	75	Endosperm
Legumes				
Broad bean	23	1	56	Cotyledons
Garden pea	25	6	52	Cotyledons
Peanut	31	48	12	Cotyledons
Soybean	37	22	12	Cotyledons
Other				
Castor	18	64	Negligible	Endosperm
Oil palm	9	49	28	Endosperm
Pine	35	48	6	Megagametophyte
Rapeseed	21	48	19	Cotyledons

Source: (Bewley et al. 2000)

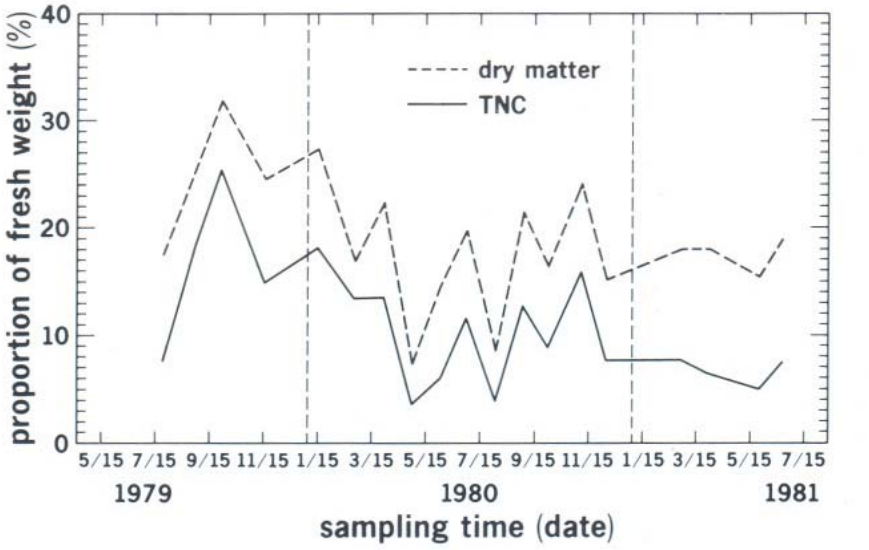

Figure 3: Seasonal variation of root dry weight and total nonstructural carbohydrates (TNC) of *Cirsium arvense*. Single samples for each collection date were analyzed (McAllister & Haderlie 1985)

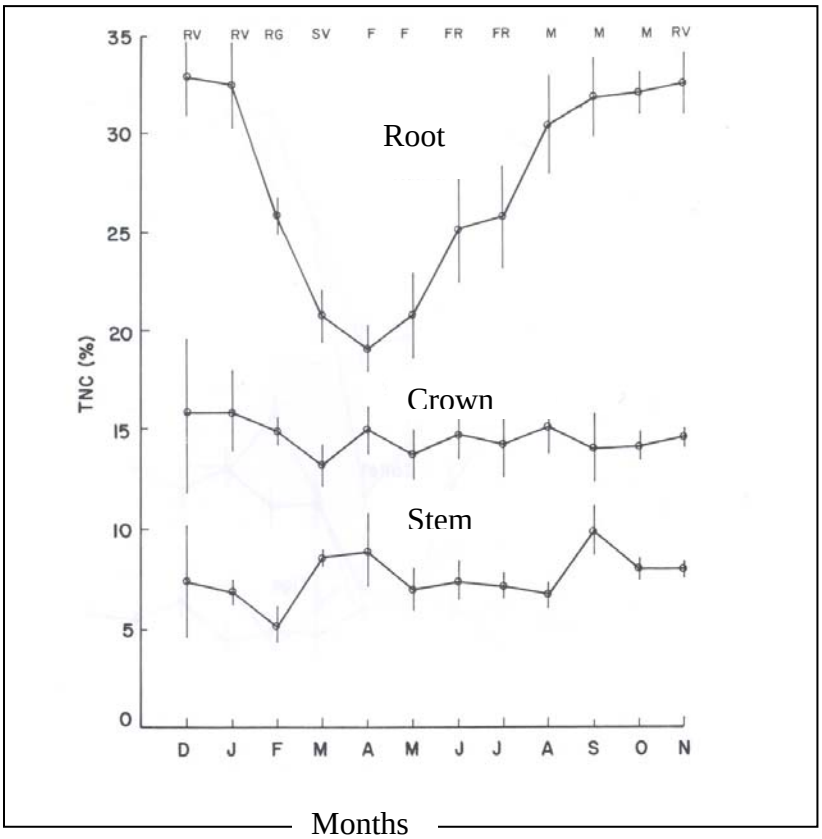

Figure 4: Changes in Total Nonstructural Carbohydrates (TNC) of *Solanum elaeagnifolium* from olive plantations. In the phenological stages, RV: dormancy, RG: regeneration, SV: vegetative stage, IF Floral initiation, F: flowering, FR: seed filling and M: maturation. Vertical bars are standard deviation. Modified from Bouhache *et al.* (1993)

2.2.2. Shoot establishment and elongation

Once the compensation point has been reached, shoots are established and can sustain their growth by autotrophy. If the required resources for growth are present, then elongation commences. Tworkoski (1992) and Gesch *et al.* (2007) have shown that shoot elongation corresponds to more photo-assimilates moving to roots than what occurs at the aerial bud, flower or post-flower stages. Studies on the partitioning of carbohydrates in relation to environmental effects in herbaceous species also have proven this increased photo-assimilate allocation (Ogden 1974; McAllister & Haderlie 1985; Wilson *et al.* 2001; Orthen & Wehrmeyer 2004; Wilson *et al.* 2006). This may suggest that carbohydrate allocations leading to growth of vegetative parts (horizontal roots, rhizomes, etc.) starts just after the establishment of shoots as observed by Zimdahl (1993) for some species.

As a consequence of this high basipetal translocation of photo-assimilates that elongates and thickens horizontal roots and rhizomes, new shoots emerge to form clonal plants. This additional reproductive ability shows physiological integration, i.e., allowing clones to share resources (Alpert 1996; Nilsson & D'Hertefeldt 2008; Peterson & Chesson 2002; Wooldridge *et al.* 1997), including defense compounds and thus systemic resistance (Gómez & Stuefer 2006; Gómez *et al.* 2007; Gómez *et al.* 2008). These studies were done in heterogeneous environments but knowledge on whether young clones in agricultural ecosystems could be affected by disturbances so that intact shoots can regulate the growth in the whole clone is still scarce.

2.2.3. Development of reproductive organs

Perennial weeds reproduce by both sexual and asexual means. Sexual reproduction is typical for most flowering plants (Batygina 2005; Bewley *et al.* 2000; Geber *et al.* 1997; Gibson 2004; Gibson 2005). The type of vegetative and carbohydrate storing organs differentiate perennial weeds (Anderson 1999) and their means for survival and spread. There is an evident lack of research on the asexual reproduction of perennial weeds (Heide 2001) even though several studies on *Eurphorbia esula* (Anderson *et al.* 2005; Choa *et al.* 2006; Gesch *et al.* 2007; Horvath 1999; Horvath *et al.* 2002; Horvath *et al.* 2006) have addressed mechanisms behind carbohydrate storage associated with asexual reproduction. Unfortunately, most of the work has focused only on sucrose and starch while studying cold acclimation. It is true that starch is a main carbohydrate reserve in plants, but Hendry (1987) estimated that 12% of angiosperms use fructans as main storage compounds. Fructans are most common in plants in temperate areas with seasonal drought or frost (Hendry 1993) and are mostly found in the *Asteraceae* (Appezzato *et al.* 2008).

According to the Raunkiær system (Mikkelsen 1968), weedy perennials with underground storage organs are classified as geophytes. The understanding of carbohydrate storage in underground organs (root, rhizome, tuber, etc.) could improve the efficacy of autumn soil cultivation because exhaustion of stored carbohydrates would weaken vegetative propagules at the sprouting period in spring. However, this would be ineffective if performed at the wrong time. If cultivation occurs a long time before

dormancy, shoots will establish and store new energy for spring sprouting. During the dormancy period, fragmentation of the underground organs (roots, rhizomes or tubers) cannot result in any shoot growth thus keeping dormant buds with enough carbohydrate reserves to allow them to sprout in spring time. Therefore, it is crucial to know and fully understand the dormancy period and its mechanisms for troublesome perennial weeds.

2.2.4. Dormancy of bud banks on vegetative propagules

Vegetative buds of geophytes are hidden in the soil (Mikkelsen 1968). As opposed to woody perennials (e.g., phanerophytes) that have buds on aerial parts, this is an avoidance mechanism to biotic or abiotic disturbances (herbivores, extreme temperatures, water deficit, etc.). Klimesova & Klimes (2006) give more details on bud bank definitions, their role and mechanisms in vegetative regeneration of herbaceous perennial plants.

Dormancy of buds of vegetative propagules depends on both external and internal factors and has been categorized into three main types: ecodormancy, paradormancy and endodormancy (Lang 1987; Anderson & Choa 2001; Rohde & Bhalerao 2007). Ecodormancy is regulated by environmental conditions such as temperature and drought (Kamenetsky 2009). Paradormancy or the correlative inhibition is controlled by the apical dominance (Choa *et al*. 2006). Endodormancy results from internal physiological factors. Although the involvement of phytohormones is known for all the types of dormancy (Choa *et al* 2006), absciscic acid (ABA) and gibberellic acid (GA) are associated with endodormancy. It is worth emphasizing that for perennials the regenerative bud set is not necessarily within a given growth stage.

Morphological and physiological characteristics of bud dormancy are still to be explored. For example, the balance of carbohydrate and other metabolites, osmotic adjustment associated with the changes of water freezing or drought or the presence of other solutes, and tissue maintenance are so far not fully understood.

2.3. Water relations and fructan

Keeping water homeostasis is invaluable for all living organisms. In contrast to abundant literature about water relations in trees and shrubs relatively few studies have addressed

herbaceous perennial plants. Munne-Bosch (2007) has published a review in which the literature on age-related water relations and photosynthesis was explored. Most of the literature currently found is limited to water relations in ornamental species (Starman & Lombardini 2006; Zollinger *et al.* 2006) of arid zones.

There is useful information from these studies that can probably be used for other herbaceous perennials. For example, the changes of the stomatal closure in relation to ABA and water status, the size of the plant, the age and the leaf area, etc. are some of the factors that affect plant physiology and morphology through water loss by transpiration. Water deficit has an effect on increased C/N ratio that increases sucrose concentration and this leads to a feedback inhibition of photosynthesis. Limiting water is also followed by drought avoidance mechanisms with the reduction of the area of high transpiration.. New growth will only be possible when water is not limiting. The involvement of water in germination, as for the sprouting has been discussed in 2.2.1. Cell to cell communication allows water and solute flow and a renewal of growth (Rinne *et al.* 2001).

Fructans, known as underground carbohydrate storage in numerous plants species, are thought to be involved in drought and freezing-tolerance (Cyr *et al.* 1990; Thomas 1991; Tworkoski 1992; Turtuliano & Figueiredo-Ribeiro 1993; Wilson *et al.* 2001; Orthen 2001; Pavis *et al.* 2001; Van Laere & Van Den Ende 2002; Wilson & Michiels 2003; Van Den Ende *et al.* 2006, Itaya *et al.* 2007). Fructans are formed by elongating sucrose with many molecules of fructose. The polymers formed can be linear (inulin) branched (levan) or both depending on the species (Vijn & Smeekens 1999, Ritsema & Smeekens 2003). In Fig. 5, the smallest inulin and levan types of fructan are illustrated. Inulin type of fructan is the best known in the *Asteraceae* whereas levan is known in cereals. Fructans accounted for 73% of total carbohydrates in *C. arvense* roots in October, while free sugars consisted of sucrose (87%), fructose and glucose (Wilson *et al.* 2006).

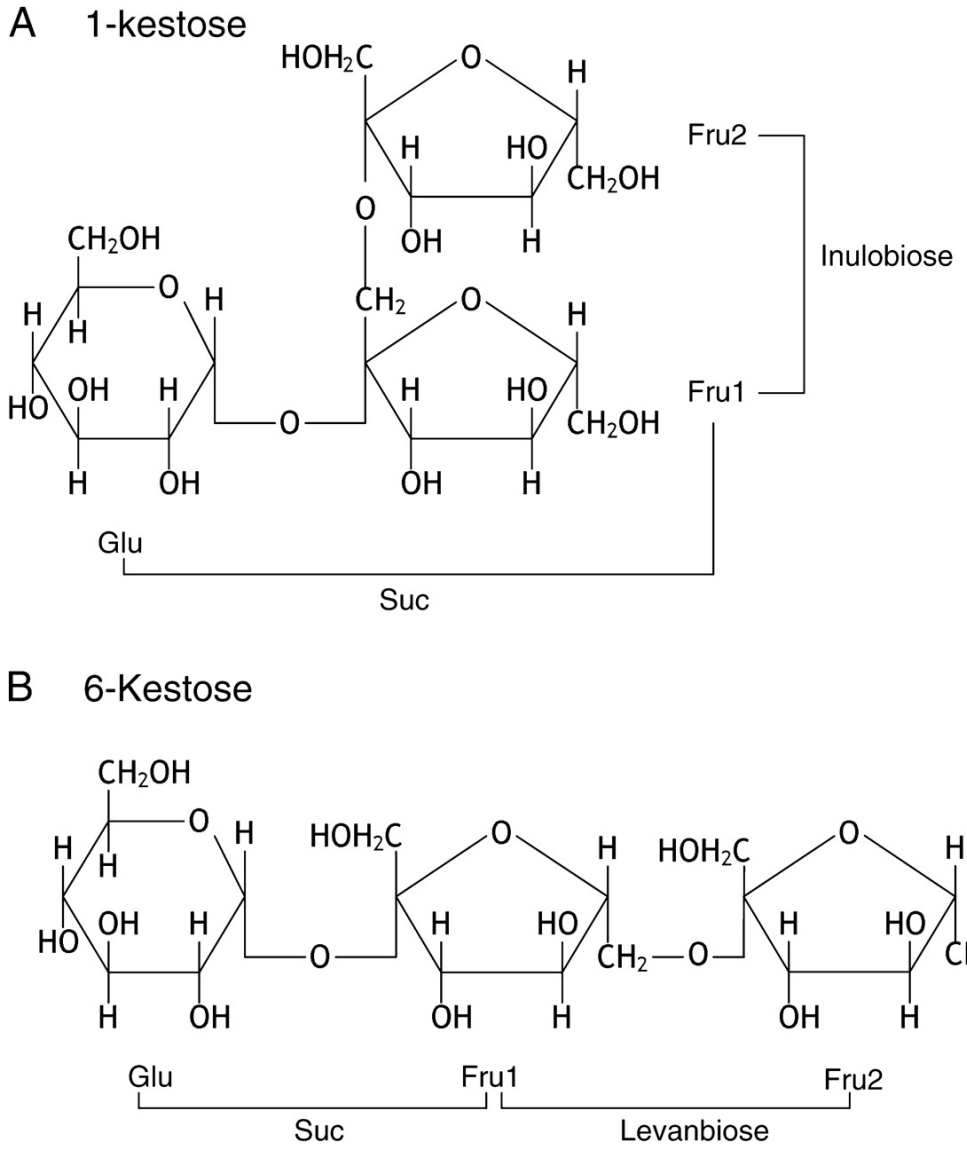

Figure 5: The structure of 1-kestose (A), the smallest inulin, and 6-kestose (B), the smallest levan-type fructan (Valluru & Van den Ende 2008).

3. Experimental work on *Cirsium arvense* and *Tussilago farfara*

In this chapter, we summarize our four experimental studies which are detailed in the attached appendices. We give a brief summary of the material and methods, present the results and discuss the main findings in light of the phenology and the source-sink dynamics of carbohydrates in relation to management of perennial weeds.

3.1. Material and methods

3.1.1. Species and planting material

In all experiments, only asexual reproduction was considered and roots of *C. arvense* and rhizomes of *T. farfara* were planted and analyzed. In paper IV, we also included *Elytrigia repens* for a comparison purpose. We used either 5 cm or 10 cm long fragments. Fragments were planted at a depth of 5 cm for all the experiments. The media used was a sphagnum sand mixture (GB-Pindstrup substrates N^{o}. 1, pH 6.0) except for the drought experiment (Paper IV) where a sandy-loam soil was used.

3.1.2. Summary of the experimental methods

Experiments were conducted in greenhouse and/or growth chambers. In the first and third studies, Papers I and III, destructive sampling was used to measure nonstructural carbohydrates. In Paper I, we also used the labeling of juvenile plants with carbon isotope (^{14}C) to track the start of the downward translocation of photo-assimilates during early growth. In the second study and the fourth studies, Papers II and IV, we used repeated measurements to evaluate photosynthesis activity (Paper II) and the soil water content (Paper IV) to evaluate their effect on weed biomass.

3.1.2.1. Determination of carbohydrate concentrations

Concentrations of starch, sucrose, glucose and fructose from roots and rhizomes were measured by HPLC based on Liu *et al.* (2004). To quantify fructan reserves, we used the degree of polymerisation (DP) as an indirect measurement obtained after acid hydrolysis

of the samples (Chatterton & Harrison 2003; Steegmans *et al*. 2004). For more details on fructan determination, see Paper I.

3.1.2.2. ^{14}C labeling and translocation measurements

Using $NaH^{14}CO_3$, enclosed potted-plant canopies were labelled (Carvalho *et al.* 2006; Hansen 1967). The exposure of the canopy $^{14}CO_2$ lasted for four hours between 9:00 and 16:00. We quantified translocation of ^{14}C in the root/rhizome system with a liquid scintillation counter (WALLAC WinSpectral 1414) after sample combustion in a sample oxidizer (Model 307 sample oxidizer, Packard).

3.1.2.3. Net photosynthesis

We used CIRAS-2 (CIRAS-2 Portable Photosynthesis System, PP SYSTEMS, 2007, Amesbury, MS, USA) to measure leaf gas exchange. To ensure that the measurements on different days were comparable, the measurements were done at 380 ppm CO_2, 20°C air temperature, 8.5 mbar of vapour pressure deficit and a light level that approached light saturation. To determine the light level, some preliminary light response curves were made on the two species at the start of each experiment (Paper II).

Before cutting the shoots, we chose a healthy leaf and baseline measurement was taken. The same leaf was used for all the measurements. Two days after disturbance, a second measurement was performed and then followed by several measurements on a weekly basis over three weeks. All measurements were done between 9:30 and 14:30

3.1.2.4. Biomasses at different soil water contents

A gradient of soil water contents was created by a cover crop, barley (*Hordeum vulgare* L., SIMBA 08T5 Øko) at different densities, in pots where roots of *C. arvense* and rhizomes of *T. farfara* and *E. repens* were planted. After 30% of the root/rhizome fragments had emerged, the drip irrigation was stopped and five measurements of soil water content were done for two weeks. Biomass of harvested shoots was taken at the end of the experiments. In addition, relative water content (RWC) in leaves was measured.

3.1.2.5. Phenotypic characteristics

Two phenological characteristics were recorded at different sampling times of the early growth experiment: the number of leaves per shoot and the total leaf area per box. Total leaf area was measured with a leaf area meter (LI-3100 Area meter, LI-COR, Lincoln, Nesbraska, USA).

3.2. Results and discussion

3.2.1. Carbohydrate dynamics during early growth

Among all the measured carbohydrates, fructose and fructan varied significantly during the course of the experiments (Table 3). At the planting time of *C. arvense* and *T. farfara*, fructose content was nearly zero mg g^{-1} (Fig. 6). But after emergence, its concentration was the highest and it decreased thereafter. While fructose increased, fructan continuously decreased for *C. arvense* to reach the minimum at the same time as fructose concentrations. However, a slight increase of fructan was observed in *T. farfara* after the planting time. Detailed results can be found in Paper I.

Table 3: Analysis of variance of free hexoses (fructose and glucose), sucrose and the degree of polymerization (DP) of fructan in planted roots/rhizomes at different sampling times during the early growth of *C. arvense* and *T. farfara*.

Species	Experiment	Carbohydrates	p- value and Significance
Cirsium arvense	I	Fructose	**0.012 ***
		Glucose	0.48 NS
		Sucrose	0.43 NS
		DP of fructan	**0.009 ****
	II	Fructose	**< 0.0001 *****
		Glucose	0.94 NS
		Sucrose	0.004 **
		DP of fructan	**< 0.0001 *****
Tussilago farfara	I	Fructose	**< 0.0001 *****
		Glucose	0.002 **
		Sucrose	0.61 NS
		DP of fructan	**0.012***
	II	Fructose	**< 0.0001 *****
		Glucose	0.33 NS
		Sucrose	0.26 NS
		DP of fructan	**0.038***

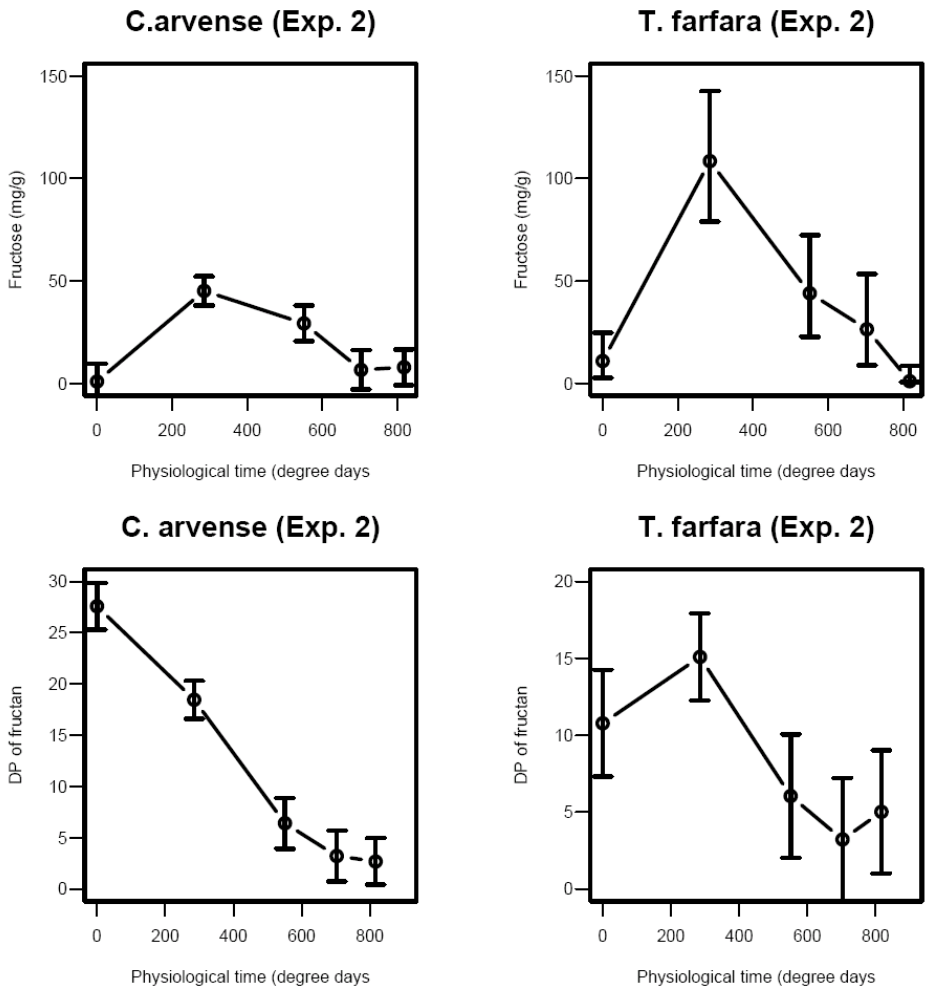

Figure 6: Variations of fructose (mg g-1 of dry weight) and fructan (degree of polymerization) from the planting time of root of *C. arvense* and rhizomes of *T. farfara* to four weeks after emergence. Only results from the second experiment are shown. The upper graphs illustrate the concentrations of fructose at different sampling times. The lower ones illustrate the decrease of the degree of polymerization of fructan as they release fructose (from Paper I). Vertical lines give 95 % confidence interval of the mean for each sampling time.

The labeling experiment showed that only three weeks after emergence (between 500 and 550 degree days) ^{14}C applied on leaf canopy was already translocated to the root/rhizome systems (Fig. 7). That time corresponds to eight leaves for *C. arvense* and six leaves for *T. farfara.*

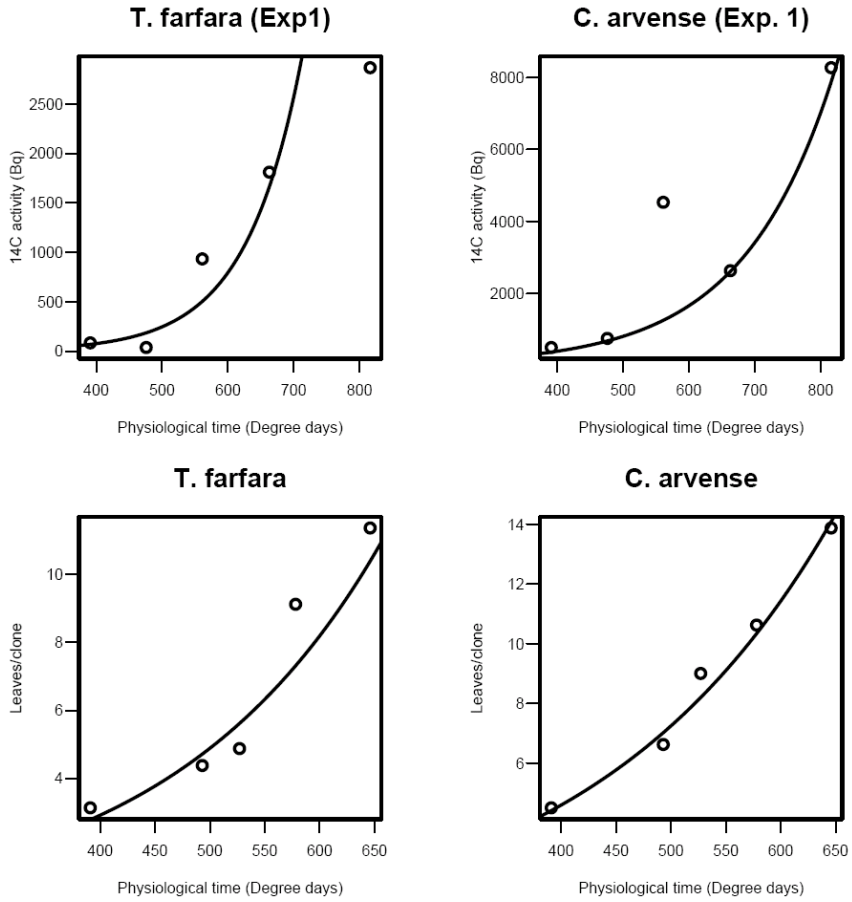

Figure 7: ^{14}C content of planted roots of *C. arvense* and rhizomes of *T. farfara* after labelling the shoot canopy from one week to four weeks after emergence (upper graphs). The number of leaves during the labelling period (lower graphs). Only data from the first experiments are shown (from Paper I).

The depletion of fructose-based reserves during early growth has been reported in earlier studies (Alexopoulos *et al.* 2009; Benkeblia 2003; Ovono *et al*. 2009; Shin *et al*. 2002; Spencer *et al.* 2001; Yasin & Bufler 2007) but the investigation on the start of basipetal translocation of photo-assimilates to depletion is new. This allowed us to be more precise on previous findings that used dry weight minimum (Gustavsson 1997) for *C. arvense*. It was revealed that basipetal translocation of photo-assimilates started before the minimum content level of carbohydrates in underground parts was reached (Fig. 6). At the planting time, the fructan amount for *T. farfara* was lower compared to that of *C. arvense*. This was in agreement with previous findings on dry matter measurements (Bakker 1960). The reason behind this could be due to the occurrence of flowering in *T. farfara*. Flowering in *C. arvense* occurs only after a period of the vegetative growth with

impact on carbohydrate partitioning (Tworkoski 1992), but this energy for flowering might come from the stored carbohydrates for *T. farfara*.

3.2.2. Photosynthetic activity of established shoots

After shoot establishment, disturbed and undisturbed clones did not show any differences in terms of photosynthetic activity (Fig. 8).

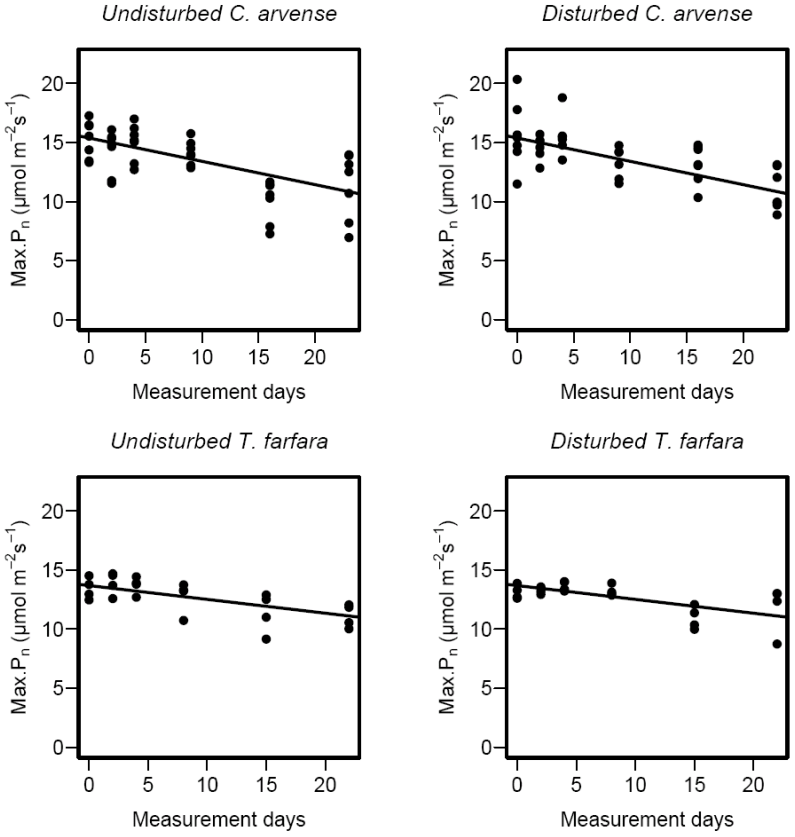

Figure 8: Maximum net photosynthesis, Max $P_{n,}$ plotted against day of measurement in 2008: Day 0 represents the baseline measurement (rosette growth stage) followed by measurements taken on disturbed clones (to the right) and on undisturbed ones (to the left). The experiment end corresponds to the bolting growth stage. The light level in the cuvette was 500 and 450 µmol m^{-2} s^{-1} for *Cirsium arvense* and *Tussilago farfara*, respectively (from Paper II).

There was no physiological integration for the two species as opposed to some rhizomatous and stoloniferous perennial species such as *Trifolium repens* L. and strawberries of the genus *Fragaria* (Alpert 1996;Wooldridge *et al.* 1997; Gómez & Stuefer 2006; Gómez *et al.* 2007; Gómez *et al.* 2008).

3.2.3. Carbohydrate accumulation

Before four weeks after planting, shoots could not store carbohydrates in the underground organs. But after four weeks fructan did not differ between underground organs of juvenile shoots and mature shoots regardless the temperature of treatment (Fig. 9). This

might suggest that establishment of shoots is expected after four weeks, on average. In both juvenile and mature shoots of *C. arvense*, the DP of fructan was related to temperature, but not for *T. farfara* (Fig. 9). With lower temperature, the accumulation becomes higher in roots of *C. arvense.* Starch was not important in carbohydrate storage (see Paper III).

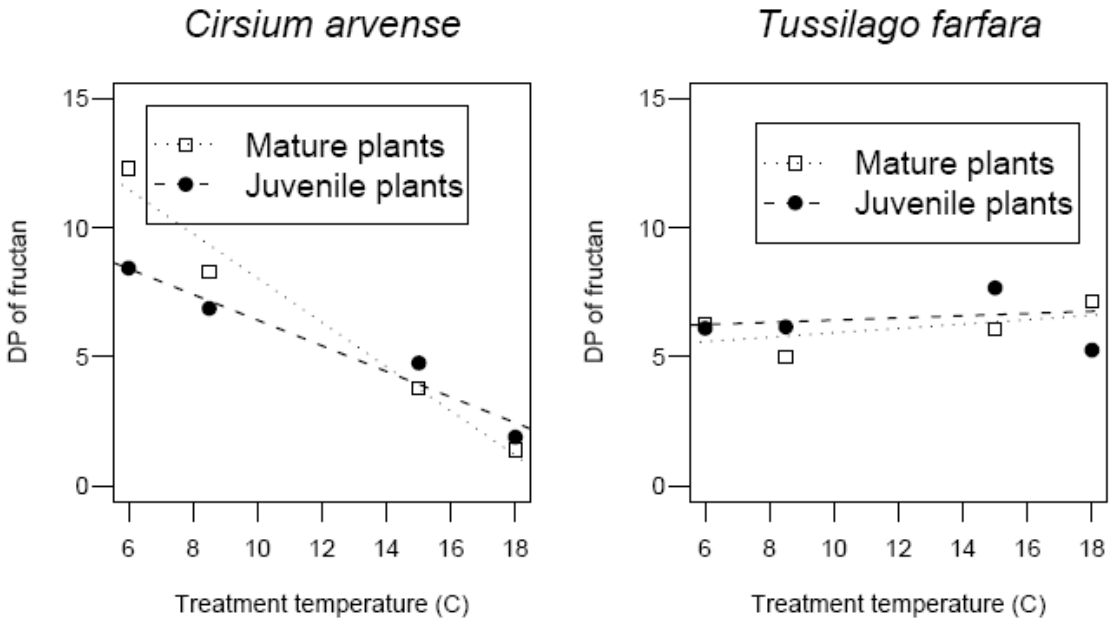

Figure 9: Degree of polymerization (DP) of fructan of mature and juvenile plants in roots of *Cirsium arvense* and rhizomes of *Tussilago farfara* treated at different temperatures (Paper III)

The different levels of carbohydrate storage highlighted in the early growth between *C. arvense* and *T. farfara* were found here. These differences in fructan concentrations may lie in the flowering initiation in *T. farfara* as already mentioned above. Part of the energy is used to promote the flowering when plants are exposed to low temperatures: vernalization. Common mechanisms that regulate flowering and dormancy have been reviewed (Horvath 2009), and low temperature impact on flowering competence was shown in crown buds of *Euphorbia esula* (Dogramaci et al. 2010).

The lack of differences in biomasses of shoots emerged from fragments taken on root systems after temperature treatment, for *T. farfara* in Table 3, is not surprising

because the carbohydrates contents were not different. However, for *C. arvense*, the biomasses did not correlate with carbohydrate storage differences. Mature plants of the first experiments did not differ in terms of shoot biomasses harvest either 15 or 28 DAE (Table 4). Roots taken from juvenile donor plants responded to the differences in carbohydrate content.

Table 4: Mean dry weight (mg) of shoots regenerated from roots of *Cirsium arvense* and rhizomes of *Tussilago farfara* and harvested 15 and 28 days after emergence (DAE). Roots and rhizomes were collected from plants grown under different temperature conditions: Low, medium and high equivalent to day/night temperatures of 7C/5C, 10C/7C and 18C/12C, respectively. Plants were of different age at the time of transfer to respective temperatures: 4 weeks old plants (juvenile) and 11-17 weeks old plants (mature). (from Paper III)

Year	Harvesting time (DAE)	Plant age at the treatment start	Treatment temperature	Estimates of dry weight (mg)	
				Cirsium arvense	*Tussilago farfara*
2008	15	Juvenile	Low	93.4 a	88.2 a
			Medium	45.9 ab	115.9 a
			High	21.7 b	30.3 a
		Mature	Low	69.7 a	132.8 ab
			Medium	51.5 a	189.9 a
			High	16.3 a	38.0 b
	28	Juvenile	Low	381.0 a	264.3 a
			Medium	605.3 a	353.5 a
			High	23.1 b	37.5 b
		Mature	Low	414.0 a	503.0 a
			Medium	298.4 a	239.3 a
			High	188.2 a	178.2 a
2009	28	Mature	Low	204.6 a	226.8 a
			High	133.9 b	264.3 a

The rate of emergence was estimated 6, 15, 22 and 28 days after the first emergence. The differences in the emergence rates of roots taken from juvenile plants either treated with lower or higher temperatures reflect the importance of carbohydrate reserves (Fig 10 & 11), fructan concentration being different at different temperatures (Fig. 9).

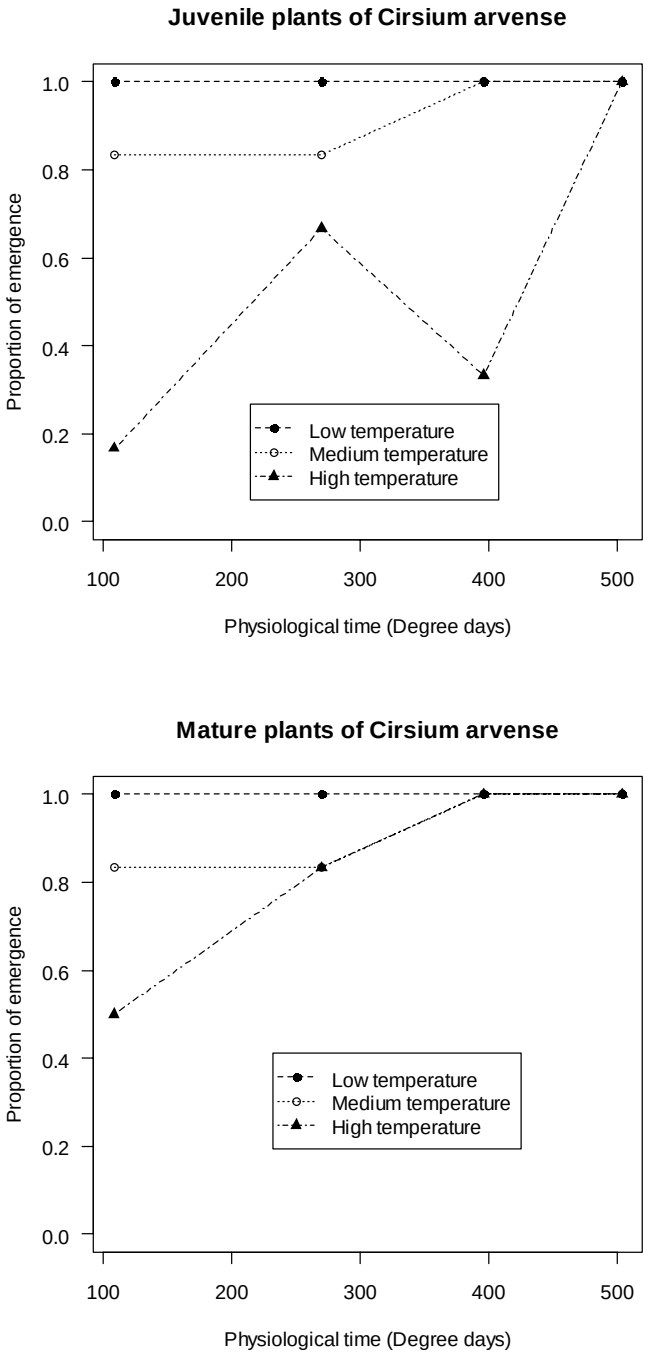

Figure 10: The emergence proportions from roots of *C. arvense* against physiological time (degree days) after the first emergence. Different symbols represent temperatures from where root fragments were obtained. The emergence rates from roots of juvenile and mature plants are illustrated by upper graph and lower graph, respectively (Paper III).

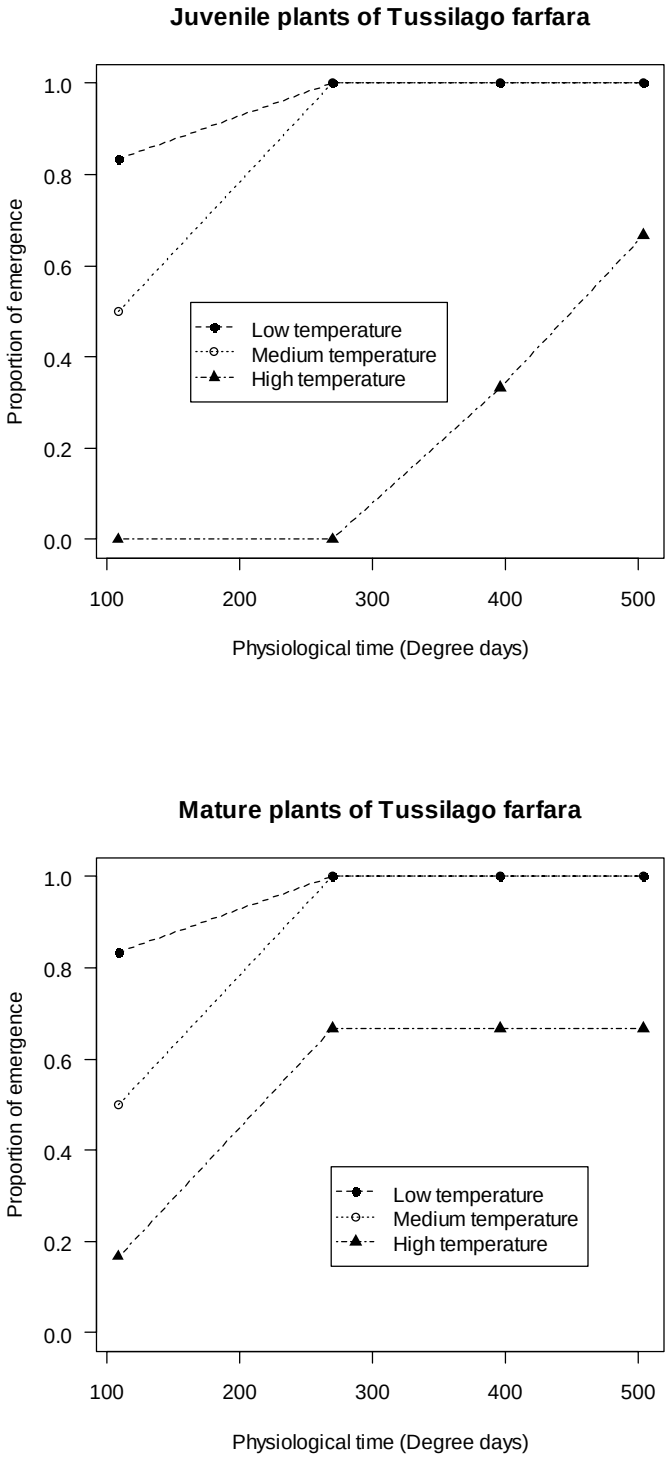

Figure 11: The emergence proportions from roots of *T. farfara* against physiological time (degree days) after the first emergence. Different symbols represent temperatures from where rhizome fragments were obtained. The emergence rates from rhizomes of juvenile and mature plants are illustrated by upper graph and lower graph, respectively (Paper III).

All roots with higher fructan content, from lower temperatures, had already emerged shoot six days after the first emergence (DAFE). The emergence of all shoots

lasted until 22 DAE for medium temperatures for *C. arvense* while the roots at high temperatures lasted until 28 DAE. Rhizomes of *T. farfara* taken from high temperature did not give visible shoots at a rate of hundred percent 28 DAE.

3.4.4. Drought effect on early growth of perennials

In broadleaf perennial weeds, *C. arvense* and *T. farfara*, it appeared that there was a relationship between the soil water content and the shoot biomass (Fig 12). The analysis of the relationships is summarized in Table 5. There was a relation between soil water content and shoot biomass for *T. farfara* in both years whereas only the first experiment showed significant correlation for *C. arvense*. No relationship was seen for *Elymus repens.*

Table 5: Analysis of the linear relationship between soil water content and the shoot biomass after 14 days of growth from roots of *Cirsium arvense* and rhizomes of *Tussilago farfara* and *Elymus repens*. ***: Very highly significant, **: Highly significant, *: Significant and NS: Non significant

Experiment year	Species	Relationship analysis		
		Intercept	slope	P-value
2009	*T. farfara*	-5.90 (1.56)	0.46 (0.09)	< 0.0001 ***
	C. arvense	-2.31 (1.42)	0.18 (0.06)	0.013 *
	E. repens	0.02 (0.31)	0.02(0.01)	0.22 NS
2010	*T. farfara*	-1.43 (1.60)	0.26 (0.09)	0.006 **
	C. arvense	0.018 (0.39)	0.04 (0.01)	0.058 NS
	E. repens	0.72 (0.2)	-0.01 (0.01)	0.08 NS

The relative water content in broadleaf weeds confirmed that *T. farfara* is less tolerant to the soil drought (Fig 13). The slope of the linear regression is steeper for *T. farfara* (0.025) than *C. arvense* (0.012) and their respective p-values are 0.008 and 0.024.

The susceptibility to low soil water content of broadleaf perennial weeds can give an opportunity window for their control. This will depend on the crop and cropping systems. It was shown that high density of barley means high competition for water resources. This is supporting the theory of high crop density to reduce weed infestation (Weiner 2001, Kristensen 2008). It might be challenging for humid temperate regions, but if justified economically, drought can be used to reduce weed infestation in vegetable cropping. In arid zones, water management can also be an opportunity to control perennial weeds in dry-seasons.

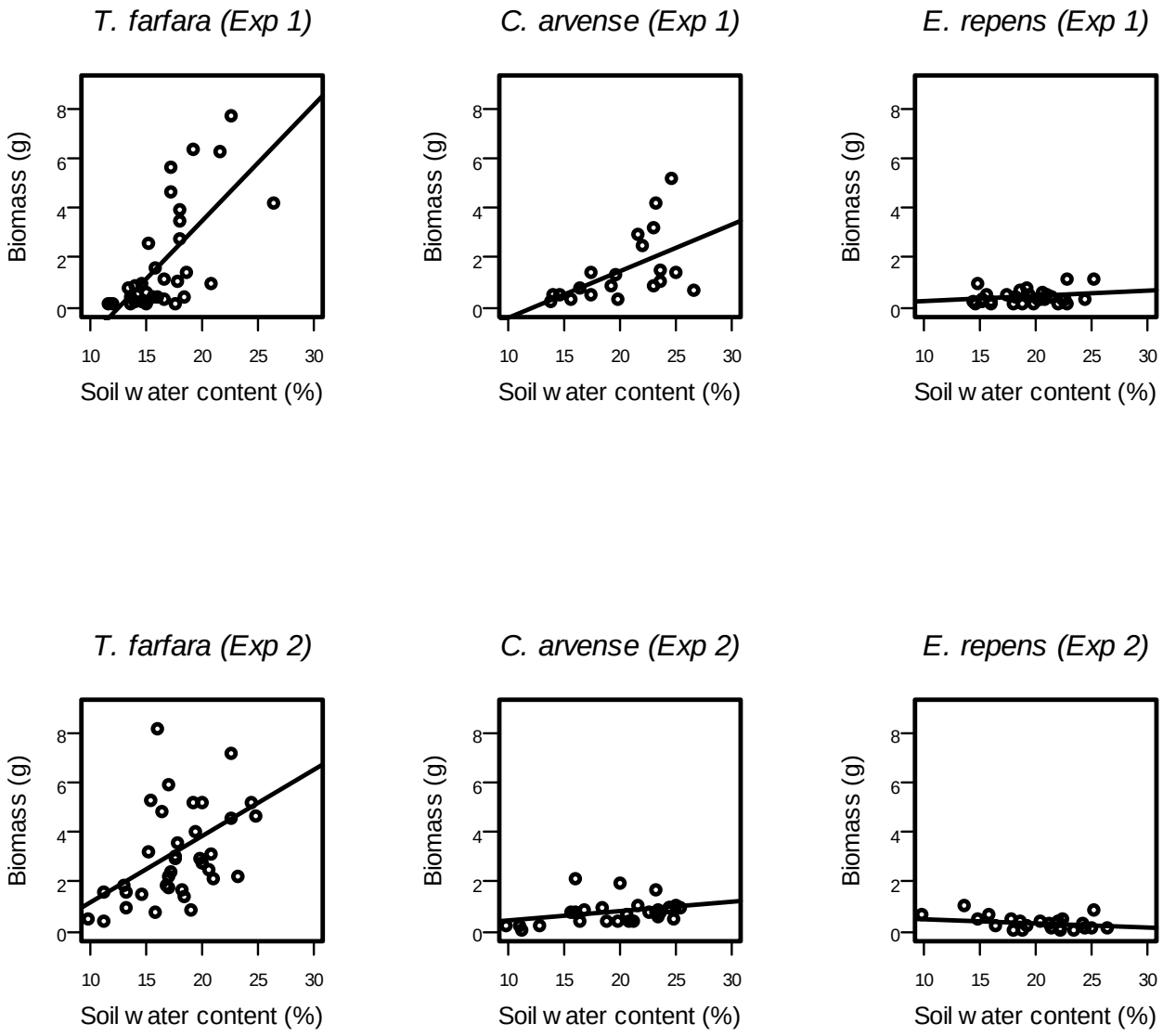

Figure 12: Relationship between soil water contents and biomass of shoots of three perennial weeds: *Tussilago farfara*, *Cirsium arvense* and *Elymus repens*. The upper graphs represent the first experiment whereas the lower ones are for the second experiment (Paper IV)

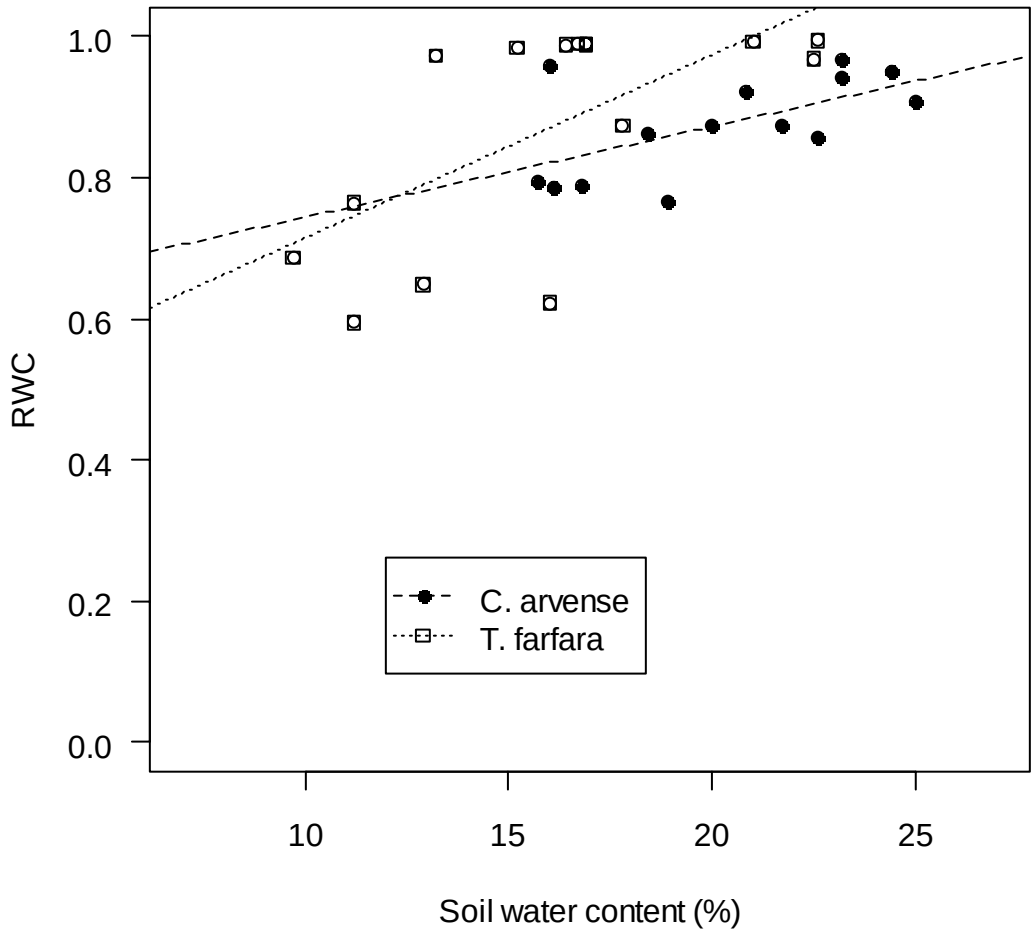

Figure 13: Relationship between soil water contents and the relative water content (RWC) in leaves of *Tussilago farfara* and *Cirsium arvense* (Paper IV)

4. Conclusions

Carbohydrate reserves in roots of *C. arvense* and rhizomes of *T. farfara* are subjected to their depletion in association with the sprouting and the early growth. This was highlighted by the release of fructose from fructan that constitutes the energy stock of geophytes at the time of reduced or absent visible growth. The stored energy is then used to give rise to new shoots from buds when external or internal conditions allow. Before the depletion of carbohydrate reserves is complete, photo-assimilates from new shoots are already being translocated to the underground organs. Subsequently, new roots and

rhizomes start to form from the original root or rhizomes strengthened by photo-assimilates through basipetal translocation. We think, therefore, that the control of these two species would be effective if operations are planned before a large amount of photo-assimilates is translocated into the underground parts. Our experiments show that this was from late in the third week to the fourth week after emergence.

In absence of control measures for *C. arvense* and *T. farfara*, until 37 days after emergence, it was shown that two to four new shoots emerged and a clone was formed. In other clonal species, the interconnectedness of shoots leads to a physiological integration as a means to sustain the whole clone. But the newly established shoots of *C. arvense* and *T. farfara* were shown to be autonomous. Therefore, clonality does not confer any benefit to these two perennial weeds as compared to annual weeds.

The main carbohydrate storage in *C. arvense* and *T. farfara* in the underground parts was fructan. Fructan accumulation starts immediately after the shoots are established and become autotrophic. Photosynthesis leads to thicker new roots and rhizomes, which give rise to new shoots. Carbohydrate storage in roots/rhizomes of juvenile plants exposed to a gradient of temperatures did not differ from those of mature plants in similar conditions. Low temperatures were associated with high storage of fructan in *C. arvense*. This is an indication that environmental conditions influence the storage of carbohydrates. Beside the fact that *T. farfara* had lower amount of fructan, its amount was constant for the temperatures to which the plants were exposed. This is probably connected to flower bud initiation under cold conditions. A part of the energy might be used for flowering. This suggests that cultural control methods that aim at depleting the underground parts of perennials as a preventive measure against infestation during the subsequent year need to be scheduled in a way that takes local temperatures into consideration. For example, low temperatures depend on the latitudes and control measures need to be scheduled in a way that takes low temperature occurrence into account.

As for the emergence of shoots from roots/rhizomes with different fructan concentrations, a timely soil cultivation to deplete carbohydrate storages is a requirement. Soil cultivation would be more effective if done three to four weeks before the expected arrival of seasonally low temperatures, which would prevent the accumulation of fructan.

In such case, new shoots might not be able to become autotrophic and develop thickness needed for the subsequent season. In the case of temperate regions, low temperatures are normally associated with dormancy and that means that studies on dormancy time of perennial weed species need to be taken into account.

Another conclusion from this study was that stored carbohydrates are not equally used during the early growth to promote the shoot development when root/rhizome fragments are exposed to different soil water contents. Dry soils have greater adverse impacts on the early growth of broadleaf rather than grass weeds. There is potential in using water relations to control perennial weeds, but this is very much dependent on the type of crop and production system.

The overall conclusion is that during the phenology of *Cirsium arvense* and *Tussilago farfara*, the earlier growth is characterized more by depletion of carbohydrates and then followed by a basipetal translocation of photo-assimilates. Minimum carbohydrate reserves are found four weeks after emergence, but the basipetal translocation begins at three weeks after emergence. The best time of control is suggested to be between 500 to 600 degree days corresponding to 6 and 8 fully developed leaves for *T. farfara* and *C. arvense*, respectively. The shoot biomass production in broadleaf perennials is lower with dry soils. The basipetal translocation starts from three weeks and continues throughout the vegetative season, and lower temperatures are favorable to carbohydrate storage in form of fructan.

5. Challenges and perspectives

The main challenge in studying the asexual reproduction of perennial weeds is the continuous development of vegetative propagules under the seasonal changes of the year. In contrast to sexual reproduction, seeds are distinct units with almost similar amounts of carbohydrates or other compounds for a given ecotype of a particular species. The storage in seeds occurs at the same time and germination occurs at the same period depending on the soil layer where the seeds are located and the environmental conditions.

Nevertheless, in the case of asexual reproduction of perennial weeds, in addition to inter- and intra-specific differences for ecotypes, bud bearing organs contain different amount of resources depending on the environmental conditions, the time of life cycle

and biotic or abiotic stress, etc. Bud bearing organs are located at different soil layers and age of fragments is different. Unexpected bud sprouting occurs when the apical meristem is disturbed: paradormancy. All these reasons contribute to the complexity of the asexual reproduction, thus perennial weeds become more difficult to study than annual weeds. This variability associated with vegetative propagules leads to a huge variation in collected data. Inherently, variability makes control strategies more difficult.

It seems to be very difficult to unravel the variations in collected data even when a single ecotype is used. Although studies in controlled environments remain important to know from where to start, large scale on-farm studies would probably address the question in a more straightforward way. Considering populations instead of selected ecotypes may portray a general picture on the regeneration of perennial weeds of economic importance. For example, in this thesis, simulating the carbohydrate dynamics have generated thoughts on some relevant questions to be addressed in the future:

- How big is the variation of carbohydrate storage under field conditions? Here consideration would be to initiate comparisons of many ecotypes of both species from different environmental conditions;
- The understanding on the regulations (e.g., enzymes) responsible for fructan synthesis and depolymerization in *C. arvense* and *T. farfara* is needed. This knowledge can be used to trigger the depletion carbohydrate storage before the unfavorable conditions and to prevent their synthesis.
- What are the relationships between dormancy of vegetative propagules, biotic or abiotic factors and carbohydrate reserves?
- It is also be important to find out if the control times here suggested correspond to the crop calendar. This matters because weed control can not be done at any developmental stage of the crop. More experiments where crops and weeds coexist are needed.

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