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Čeleď *Fabaceae* - kořenové systémy

Doktorská disertační práce

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OBSAH

SUMMARY

This PhD thesis focused on the root systems and the nodulation within the family *Fabaceae* (legume family). Legumes are known for their symbiotic relationship between their root systems and *Rhizobium* s. l. bacteria. This association enables these plants to utilize aerial N_2, which is inaccessible to many other species. The most important forage plants belong to this family.

The thesis consists of five parts. In the first part the intra- and interspecific variability of root systems of 21 legume species were investigated in the field. Root systems and nodules of individual species as well as environmental influences on root morphology were described. It became apparent that the root systems of common European legumes are highly variable. The variability of root systems was attributed to Raunkiear´s life forms rather than the membership of species in tribes.

The second and third part of the thesis examined the influence of nutrient availability (NPK) on haresfoot clover (*Trifolium arvense*) and zigzag clover (*T. medium*) in pot experiments with alkaline soil. First it is shown that the acidicole behaviour of *T. arvense* is based on P limitation when growing on alkaline soil. The relatively large root system of *T. arvense* in treatments with P contrasted highly with the poorly developed root systems in other treatments. Second it is demonstrated that *T. medium* can be recommended as a suitable legume species for cultivation on alkaline soils. It required a relatively high P and K supply as well as additional mineral N supply to achieve maximum growth potential. The mineral N requirements of *T. medium* were substantially higher than of *T. arvense* planted in the same soil.

The thesis is completed with an investigation of two of the most important forage legumes, namely alfalfa (*Medicago sativa*) and red clover (*T. pratense*). Both species were compared at sites with organic farming and different soil texture. The herbage yield from the second and third cut was significantly affected by site. The biomass of belowground organs was not significantly affected by site or by time. *Medicago sativa* created less nodules and lateral roots than *T. pratense*. Root branching of both species was slightly higher at sites with a higher sand content. More branched roots created more nodules. The number of nodules per plant decreased during the season.

The thesis provides important insights into root traits of legumes and their ability of nitrogen fixation. In light of future challenges to agriculture, like e.g. scarcity of resources and land, climate change and biodiversity loss, the utilization of ecosystem services will gain more attention. Results of this work are thus not only interesting from a scientific perspective but also from a practical agronomic point of view. Organic as well as conventional farmers can use these findings in the future to increases benefits from legume crops and improve their overall herbage yields.

Keywords: Legumes, nodulation, nutrient availability, soil texture, species variability.

SHRNUTÍ

Tato disertační práce se zabývá kořenovými systémy a nodulací u rostlin z čeledi *Fabaceae* (Bobovité). Rostliny této čeledi, často označované též jako leguminózy, jsou schopné díky symbiotickému vztahu mezi svými kořenovými systémy a bakteriemi rodu *Rhizobium* s. l. poutat vzdušný dusík, který je pro většinu druhů rostlin nedostupný.

Práce se skládá z pěti částí. V první části byla pozorována vnitrodruhová a mezidruhová variabilita kořenových systémů 21 druhů čeledi *Fabaceae* na krajinné úrovni. Byly popsány kořenové systémy spolu s hlízkami jednotlivých druhů a také vlivy prostředí ovlivňující jejich morfologii. Kořenové systémy byly morfologicky velmi variabilní. Jejich variabilita byla dána především životní formou dle Raunkiaera, méně pak tribovou příslušnosti druhu.

Druhá a třetí část práce byla zaměřena na vliv dostupnosti živin (NPK) z hlediska kořenových systémů. *Trifolium arvense* (jetel rolní) a *T. medium* (jetel prostřední) byly zkoumány v rámci nádobového pokusu se silně alkalickou půdou. Druh *T. arvense* utvářel pouze ve variantách s aplikací fosforu relativně rozsáhlé kořenové systémy. Jeho acidofilní chování bylo vysvětleno neschopností přijímat dostatek fosforu v alkalických půdách. Oproti tomu *T. medium* se projevil jako potenciálně vhodný druh leguminózy pro pěstování na alkalických půdách. Ve srovnání s *T. arvense* vyžadoval *T. medium* pro dosažení maximálního růstu, kromě zvýšené dostupnosti fosforu a draslíku, také zvýšenou dostupnost minerálního dusíku.

Práce byla završena studií jedněmi z nejvýznamnějších pícnin - *Medicago sativa* (tolice setá) a *Trifolium pratense* (jetel luční). Teoretické poznatky kořenových systémů byly tímto způsobem otestovány v praxi. Na ekologicky obhospodařovaných zemědělských plochách s odlišnou zrnitostí půdy byly jmenované druhy hodnoceny v jetelovinotravní směsi. Hodnoceny byly nejen podzemní a nadzemní části rostlin, ale hodnocen byl také výnos a podzemní biomasa směsi. Výnos nadzemní biomasy klesal v průběhu sezóny. Při druhé a třetí seči se výnos lišil v závislosti na půdních podmínkách. Oproti tomu podzemní biomasa nebyla průkazně ovlivněna půdními podmínkami ani sezónním vývojem. *Medicago sativa* utvářela méně větvené kořeny než *T. pratense*, což se pravděpodobně projevilo i menším množstvím hlízek na nich. Více větvené kořeny obou druhů byly sledovány na půdách s větším podílem písčité frakce. Počet hlízek a jejich aktivita klesala v průběhu vegetační sezóny.

Práce poskytuje důležité informace o kořenových systémech leguminóz a o jejich schopnosti fixace dusíku. S ohledem na budoucí výzvy zemědělství (např. nedostatek zdrojů a zemědělské půdy, změny klimatu, snížení biodiverzity aj.) nabývají právě tyto poznatky na úrovni ekosystému stále většího významu. Výsledky této práce jsou zajímavé nejen z hlediska vědeckého, ale i z praktického agronomického hlediska. Ekologičtí i konvenční zemědělci mohou tyto poznatky využívat v budoucnu k celkovému zlepšení výnosů píce.

Klíčová slova: Dostupnost živin, druhová variabilita, leguminózy, nodulace, půdní textura.

1. ÚVOD A CÍLE DISERTAČNÍ PRÁCE

Tato disertační práce se zabývá morfologií kořenových systémů čeledi *Fabaceae* Lindl. (Bobovité). Rostliny čeledi *Fabaceae* (často označované též jako leguminózy) jsou schopné díky svému symbiotickému vztahu s bakteriemi rodu *Rhizobium* s. l. poutat vzdušný dusík. Nodulace (tvorba hlízek), jež je tímto vztahem vyvolána, byla zahrnuta do této studie jako součást kořenových systémů.

Fixace vzdušného dusíku čeledi *Fabaceae* je důležitým článkem koloběhu dusíku a klíčovým faktorem v zemědělské výrobě, především v rámci ekologického zemědělství. Leguminózy jsou díky svým rozsáhlým kořenovým systémům také významným zdrojem humusu v půdě. Pěstují se často jako meziplodiny, které poskytují snadno rozložitelnou organickou hmotu podporující biologické procesy v půdě. Kořeny leguminóz také chrání půdu před erozí a mají významný meliorační vliv. Díky svému rozložení v půdním profilu působí jako drobné drenáže a tím umožňují pohyb vzduchu, vody a živin v půdě. Hluboce kořenující druhy tak zpřístupňují živiny z větších hloubek a zlepšují půdní strukturu. Rostliny čeledi *Fabaceae* hrají důležitou roli v úrodnosti půdy a podporují svými kořeny výnos následných plodin v osevním postupu. Z výše uvedených skutečností vyplývá důležitost výzkumu morfologie kořenových systémů čeledi *Fabaceae*, nejen z hlediska nových teoretických poznatků, ale i pro jejich praktické využití.

Výzkum kořenových systémů je časově i fyzicky náročný. Z tohoto důvodu je většina kořenových studií zaměřena především na různé druhy trav, jejichž kořeny nedosahují takových hloubek a rozměrů. Velká pozornost je věnována také zemědělským plodinám. Mezi těmito studiemi nalezneme jen málo prací provedených na krajinné úrovni, které by poukazovali na variabilitu kořenových systémů běžných druhů leguminóz (viz. ***Článek I***).

Morfologie kořenových systémů je dána geneticky a zároveň je ovlivněna faktory prostředí. K těmto faktorům patří mimo jiné dostupnost živin v půdě (dusík, fosfor, draslík – NPK), půdní reakce (viz. ***Článek II*** a ***Článek III***) a fyzikální vlastnosti půdy (viz. ***Článek IV*** a ***Článek V***). Tyto faktory jsou jedněmi z nejdůležitějších podmínek prostředí určující úrodnost půdy a výnos plodin v zemědělství. Kořenové systémy, jak již bylo zmíněno, ovlivňují výnos plodin a jsou v přímém vztahu s nadzemními částmi rostlin, z tohoto důvodu se předložená práce zabývá i nadzemními částmi rostlin, příp. jejich biomasou tak, aby výsledky mohly být zařazeny do širšího kontextu.

Přehled cílů práce

I. Variabilita kořenových systémů běžných druhů leguminóz ve střední Evropě (*Článek I*)

Výzkum na krajinné úrovni (Plzeňsko a Rakovnicko)

Úvodní práce se zaměřuje na variabilitu kořenových systémů 21 druhů (5 tribů) čeledi *Fabaceae* na 27 stanovištích. Kořenové systémy spolu s jejich nodulací jsou porovnávány na základě odlišných životních forem druhů (RAUNKIAER 1934). Zároveň je poukázáno na faktory prostředí ovlivňující jejich morfologii.

Hlavními otázkami této části práce jsou:

a) Jak se liší morfologie kořenových systémů spolu s jejich hlízkami mezi jednotlivými druhy rostlin?

b) Jaké znaky kořenů a hlízek jsou charakteristické pro jednotlivé druhy rostlin?

II. Vliv dostupnosti dusíku, fosforu a draslíku na vzcházivost, nodulaci a růst acidofilního druhu *Trifolium arvense* L. v alkalické půdě (*Článek II*)

Výzkum v rámci nádobového pokusu
(Výzkumný ústav rostlinné výroby, Praha)

Dostupnost živin je ovlivňována hodnotami pH půdy. Acidofilní (kalkofobní) chování *T. arvense* je dáno neschopností přijímat dostatek fosforu v alkalických půdách. Tento fakt však nebyl nikdy z pohledu kořenových systémů experimentálně studován.

Hlavními otázkami této části práce jsou:

a) Jak ovlivňuje dostupnost živin vzcházivost druhu *T. arvense* v alkalické půdě?

b) Jaký vliv má dostupnost živin na kořenový systém druhu *T. arvense* v alkalické půdě?

c) Jak ovlivňuje dostupnost živin nadzemní části druhu *T. arvense* v alkalické půdě?

III. Vliv dostupnosti dusíku, fosforu a draslíku na vzcházivost, nodulaci a růst druhu *Trifolium medium* L. v alkalické půdě (*Článek III*)

Výzkum v rámci nádobového pokusu
(Výzkumný ústav rostlinné výroby, Praha)

Vliv dostupnosti živin v alkalických půdách nebyl dostatečně prozkoumán také u druhu *T. medium*. Zvolené druhy *T. arvense* a *T. medium* byly vybrány záměrně tak, aby patřily taxonomicky do stejného rodu a lišily se svými životními formami a strategiemi. U těchto dvou druhů s odlišnými stanovištními nároky byly hodnoceny nejen kořenové systémy, ale i nadzemní části rostlin a jejich vzcházivost.

Hlavními otázkami této části práce jsou:

a) Jak ovlivňuje dostupnost živin vzcházivost, růst nadzemních a podzemních orgánů druhu *T. medium* v alkalické půdě?
b) Je možné pěstovat druh *T. medium* na alkalických půdách?

IV. Sezónní vývoj biomasy jetelovinotravní směsi a vývoj nad- a podzemních orgánů druhu *Medicago sativa* na rozdílných půdách (*Článek IV*)

Výzkum na reálných ekologicky obhospodařovaných zemědělských plochách (Technische Universität München, Bavorsko)

Vliv půdní zrnitosti na výnos zemědělských plodin se těší velké pozornosti. Výnos plodin je ovlivněn kořenovými systémy. K vyhodnocení vlivu zrnitosti půdy na výnos směsi, kořenovou biomasu směsi a kořenové systémy druhu *M. sativa* bylo nutné zohlednit i další faktory prostředí.

Hlavními otázkami této části práce jsou:

a) Jaký je vliv zrnitosti půdy a průběhu vegetační sezóny na nad- a podzemní biomasu jetelovinotravní směsi?
b) Jaký je vliv půdy a průběhu vegetační sezóny na nad- a podzemní orgány druhu *M. sativa*?

V. Sezónní vývoj nad- a podzemních orgánů druhu *Trifolium pratense* v jetelovinotravní směsi na rozdílných půdách (*Článek V*)

Výzkum na reálných ekologicky obhospodařovaných zemědělských plochách (Technische Universität München, Bavorsko)

Zcela identický výzkum jako u druhu *M. sativa* proběhl také u druhu *T. pratense*. Byly hodnoceny nadzemní i podzemní části rostlin na stejných stanovištích tak, aby tyto dva zemědělsky významné druhy mohly být porovnány.

Hlavními otázkami této části práce jsou:

1. Jaký je vliv zrnitosti půdy a průběhu vegetační sezóny na nad- a podzemní orgány druhu *T. pratense*?
2. Jak se liší vývoj druhů *M. sativa* a *T. pratense* za stejných stanovištních podmínek a ve stejném termínu?

2. LITERÁRNÍ REŠERŠE

Čeleď *Fabaceae* Lindl.

Syn.: *Leguminosae* Juss., *Papilionaceae* Giseke - motýlokvěté, *Viciaceae* Adans. - vikvovité

Čeleď *Fabaceae* tvoří spolu s čeledí *Mimosaceae* (citlivkovité) a *Caesalpiniaceae* (sapanovité) řád *Fabales* (*Leguminosae*) - bobotvaré, luštinaté (AICHELE ET GOLTE-BECHTLE 2005). Čeleď *Fabaceae* je třetí nejpočetnější čeledí cévnatých rostlin. Je zastoupena více než 650 rody a čítá přes 18 000 druhů rozšířených po celém světě (DOYLE 2001). V Evropě se leguminózy rozdělují do osmi tribů (POLHIL 1981). V tropech a subtropech jsou zastoupeny dřevinnými typy rostlin, v chladnějších oblastech bylinami (SLAVÍK 1995). Přibližně 6,5 procenta rostlin čeledi *Fabaceae* vyskytujících sev České republice jsou nepůvodními druhy (MLÍKOVSKÝ ET STÝBLO 2006).

V předložené práci je pozornost věnována 21 druhům leguminóz při porovnávání druhové a mezidruhové variability. Pro výzkum vlivu určitých faktorů na kořenové systémy byly dále zvoleny čtyři druhy - *T. arvense*, *T. medium*, *M. sativa* a *T. pratense*.

Morfologie

Stromy, keře a byliny mají typické střídavé, složené, zpeřené nebo trojčetné listy, často zakončené úponkou nebo hrotem. Palisty jsou vytrvalé, někdy přeměněné v trny nebo asimilační útvary. Květy jsouuspořádány v hroznovitých květenstvích, hlávkách nebo v hroznech - často jednostranných, koncových nebo úžlabních (SLAVÍK 1995, VOLF 1988).

Květ je pětičetný. Charakteristickými částmi květu jsou pavéza (*vexillum*) - větší horní korunní lístek, nazpět ohnutý nebo orientovaný vpřed, někdy odlišně zbarvený; křídla (*alae*) - dva postranní lístky a člunek (*carina*) - dva spolu srůstající dolní lístky, ukrývající tyčinky a pestík. Někdy korunní plátky srůstají ve spodní části v trubku, kalich je srostlý. Plodem je lusk (AICHELE ET GOLTE-BECHTLE 2005).

Význam

Leguminózy jsou významným zdrojem půdního dusíku a humusu s dobrou sorpční schopností pro vodu i živiny. Působí fytosanitárně. Pomáhají zlepšovat chemické i fyzikální vlastnosti půdy. Podporují výnos plodin následných v osevním postupu a napomáhají zvyšovat úrodnost půdy (MÜLLER-SÄMANN ET KOTSCHI 1994, FLORES ET AL. 1997, LAMB ET AL. 2000).

Fixace vzdušného dusíku je důležitým článkem koloběhu dusíku a klíčovým faktorem v zemědělské výrobě, především v rámci ekologického zemědělství (CREWS ET PEOPLES 2003). Biologická fixace dusíku je hlavním mechanismem, kterým se získává dusík z nedostupné formy pro většinu organismů. Leguminózy tak ovlivňují úrodnost půdy, podporují růst rostlin a jejich minerální výživu. Minerální dusík se dostává do půdy v rámci rhizosféry leguminóz (TA ET AL. 1986), dále při rozkladu kořenů a hlízek během vegetačního období (RUSSELLE ET AL. 1994) a díky přímému propojení mezi travami a leguminózami pomocí mykorrhizních hub (HAYSTEAD ET AL. 1988).

Rostliny čeledi *Fabaceae* mají významný meliorační vliv z důvodu rozložení kořene, které působí jako drobné drenáže, jež umožňují pohyb vody i vzduchu v půdě (HNILIČKA ET HNILIČKOVÁ 2002). Leguminózy díky svým rozsáhlým kořenovým systémům přijímají živiny i z větších hloubek půdy a umožňují tak jejich zpřístupnění i ostatním rostlinám. Působí příznivě na provzdušnění a biologické oživení půdních horizontů. Napomáhají oživit půdu a zlepšit její strukturu. Na orné půdě jsou leguminózy jedněmi z nejlepších protierozních plodin. Víceleté porosty jetelovinotravních a jetelotravních směsek přispívají také k omezení plevelů.

Jetelovinotravní směsi a jetelotravní směsi

Jetelovinotravní směsi (směs trav a vysoce produkčních jetelovin, např. *M. sativa*) a jetelotravní směsi poskytují vyšší ekologickou a produkční stabilitu společenstev oproti monokulturám (HØGH-JENSEN ET SCHJØRRING 1997). Tento velmi dobře zapojený porost je odolný vůči zaplevelení a šíření chorob.

Kombinace druhů čeledi *Fabaceae* s druhy trav využívá efektivního čerpání vláhy a živin z celého půdního profilu, kdy *Fabaceae* díky svým kořenům odebírají vláhu i živiny (především P, Ca, Mg) z hlubších vrstev půd (PEOPLES ET CRASWELL 1992). Trávy čerpají naopak vláhu a živiny, především N a K, z povrchových vrstev půdy (THOMAS 1995). Dusík poskytovaný leguminózami je tedy ihned travami přijat z povrchových vrstev půdy (LEDGARD ET STEELE 1992). Víceleté leguminózy, které jsou součástí směsí, fixují více vzdušného dusíku než v monokulturách (LOISEAU ET AL. 2001, CARLSSON ET HUSS-DANELL 2003, NYFELER ET AL. 2011). Tyto směsi mohou poskytovat vyšší výnosy než nejlepší monokultury (HALLING ET AL. 2002, GIERUS ET AL. 2012, FIN ET AL. 2013).

Kořen a kořenové systémy

Kořenová terminologie

Theofrastos (370 - 285 př. n. l.), žák Aristotela, užil jako první v rostlinné terminologii pojem kořen. Ačkoliv za kořen (obr. 1) považoval všechny podzemní části rostliny, dobře si uvědomoval podobnost některých částí se stonky.

V organografii je kořen popsán jako nečlánkovaný osový orgán (*organum axiale*) cévnatých rostlin, vyvíjející se u semenáčků nahosemenných a dvouděložných rostlin jako geotropický protipól prýtu nebo u jednoděložných rostlin a kapraďorostů jako produkt adventivních růstových základů v pletivech prýtu (JENÍK 2005). V současné době se používá především označení „kořenový systém", kterým jsou nazývána (bez ohledu na morfogenezi) návazná rozvětvení jedné kořenové osy, ať už primární nebo adventivní (TROLL 1967, GROFF ET KAPLAN 1988).

Kořeny jsou většinou uloženy pod povrchem půdy a nenesou listy, tím se liší od stonků. Postranní kořeny se utváří proražením parenchymové kůry z hlavního kořene. Kořeny se ale mohou utvářet i na prýtech, zvláště pak na podzemních stoncích - tzv. adventivní kořeny (ČERNOHORSKÝ 1954). Tyto náhradní kořeny se mohou tvořit také na listech, stoncích nebo kdekoliv v místě poranění (KUBÁT ET AL. 1998, CHMELÍKOVÁ 2009).

Podzemní stonky se u nás rozlišují (dle německé terminologie) na oddenky (rhizomy) a výběžky (výmladky). Oddenky jsou charakteristické krátkými přírůstky a jsou zásobními orgány. Výběžky jsou dlouhé a tenké. Zajišťují vegetativní rozmnožování (KLIMEŠOVÁ 2006). Rozpoznání kořene od oddenku je možné podle anatomické stavby. Kořeny nejsou článkované a nemají šupiny, přesto nalézáme v literatuře mnoho nejasností (KLIMEŠOVÁ 2001).

Kořenový systém utváří obnovovací pupeny, které podporují vegetativní regeneraci. Schopnost tvorby těchto pupenů není u rostlin příliš častá. Méně než 10 % středoevropských bylinných druhů vytváří tyto pupeny a odnožuje z nich (KLIMEŠOVÁ ET KLIMEŠ 2005). Některé druhy odnožují běžně, jiné pouze po poranění (regenerativní odnožování).

Kořenové systémy lze z pohledu vegetativního rozmnožování rozdělit na několik typů, které jsou součástí klonální databáze rostlin CLO - PLA (KLIMEŠOVÁ ET DE BELLO 2009).

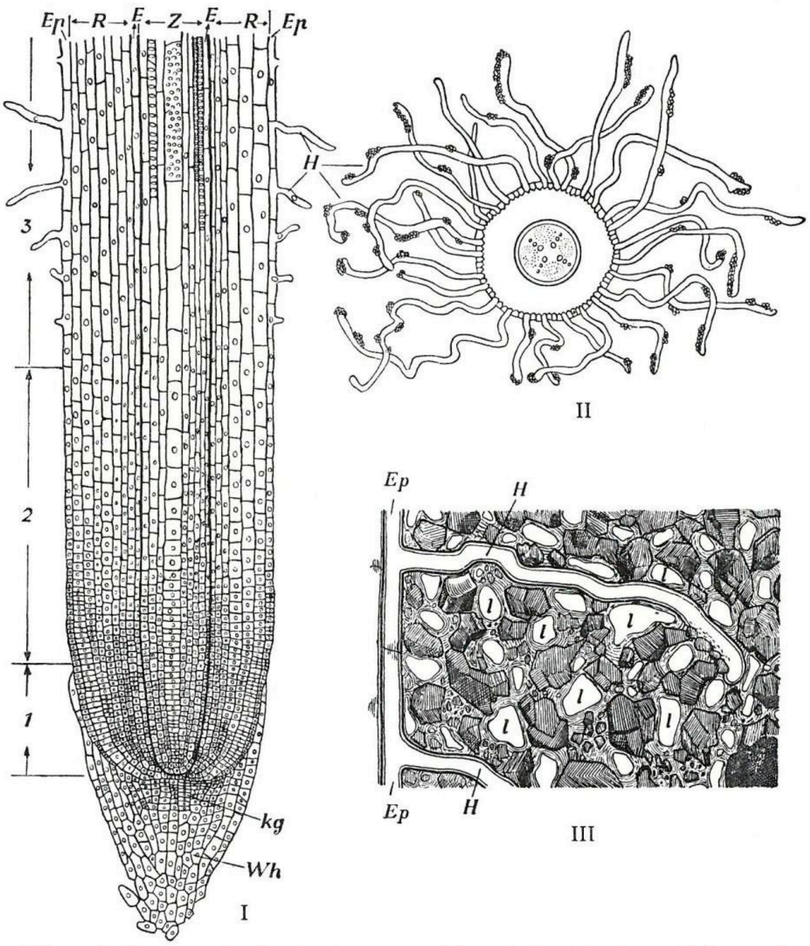


Abb. 2. I Längsschnitt durch eine junge Wurzel der Gerste. 1 Zellvermehrungszone, von der Wurzelhaube Wh geschützt, kg deren Kalyptrogen. 2 Zellstreckungszone, 3 Zelldifferenzierungszone mit Wurzelhaaren H. Z Zentralzylinder, E Endodermis, R Rinde, Ep Epidermis. II Querschnitt durch die Wurzelhaarzone. Die Haare sind mit Erdpartikelchen verhaftet. III Verlauf der Wurzelhaare im Erdboden. Schwarz von Wasserhäutchen (dünne Linien) umgebene Erdpartikelchen, l mit Luft erfüllte Hohlräume. (I n. Holman und Robbins, II n. Frank, verändert n. Rauh, III n. Sachs, alle aus Rauh).

Obr. 1: I. Podélný řez kořene, II. Příčný řez kořenového vlásku, III. Kořenový vlásek v půdě (převzato z Kutschera 1960).

Morfologie kořenových systémů

Znalosti morfologie kořenů umožňují odhadnout rostlinné nároky na vodu a živiny, současně vypovídají o schopnosti rostliny přizpůsobit se půdním podmínkám (Marschner 1995, Synman 2005). Morfologie kořenových systémů bývá často charakterizována kořenovým větvením, kořenovými délkami, kořenovými vlásky a růstem kořene do hloubky (Mengel et al. 2001). Tato práce se zabývá všemi těmito parametry s výjimkou kořenových vlásků, které jsou zde však nepřímo zastoupeny nodulací.

Morfologie podzemních orgánů je dána geneticky a zároveň je ovlivněna faktory prostředí. Mezi tyto faktory patří dostupnost světla (Koukoura et al. 2009), teplota (CANADELL ET AL. 1996), dostupnost vody (MERRILL ET AL. 2002, SKINNER ET COMAS 2010) a její podzemní hladina (LUCERO ET AL. 1999), dostupnost živin v půdě (HOWIESON ET AL. 2011, LI ET AL. 2011, CHMELÍKOVÁ ET HEJCMAN 2012B), půdní reakce (TYLER 2003, MOREIRA ET FAGERIA 2010), hustota vegetace a vegetační pokryv (HAKL ET AL. 2011), fyzikální vlastnosti půdy - půdní textura (SALAKO ET AL. 2002, NURUZZAMAN 2005, SADRAS ET AL. 2005), kořenová konkurence (OLDE VENTERINK ET GÜSEWELL 2010, HAKL ET AL. 2012A), půdní makro- a mikrofauna (GORMSEN ET AL. 2004) a další. Dokonce i geografický původ rostliny se projevuje ve stavbě kořenového systému (POLOMSKI ET KUHN 1998). Morfologie kořenových systémů je také učena druhem rostliny, životní formou (RAUNKIAER 1934) a životní strategií (GRIME 2001).

Mezi kořenovými systémy a půdou dochází ke vzájemné interakci - kořeny jsou ovlivňovány vlastnostmi půdy a půda je naopak ovlivňována kořeny (GREGORY 2006).

Disertační práce se dále zaměřuje na vliv taxonomické příslušnosti a životní formy druhu (RAUNKIAER 1934), vliv dostupnosti živin, půdní reakce a půdní textury.

Kořenová plasticita

Výsledná stavba kořenového systému je odezvou na podmínky prostředí. V této souvislosti se zavádí pojem kořenová plasticita, jako schopnost genotypu reagovat v různém prostředí utvářením odlišných fenotypů (BRIGGS ET WALTERS 1997, HODGE 2004, ŠMILAUEROVÁ ET ŠMILAUER 2010). Plasticita u pomalu rostoucích rostlin je větší než u rychle rostoucích. U jednoletých rostlin může dokonce docházet k lokální adaptaci (GALLOWAY ET FENSTER 2000). Plasticita hraje důležitou roli v reakci kořenů na půdní heterogenitu, především z hlediska živin.

Jednotlivé znaky kořenových systémů se liší v míře své plasticity. Větvení reaguje více na podmínky prostředí a je v porovnání s průměrem kořenového krčku více plastické (CHMELÍKOVÁ ET HEJCMAN 2012A). Také hloubka kořenění je v odlišných prostředích ovlivněna do různé míry prostředím a genetikou (HODGE 2004). Míra plasticity a dědičnosti se druhově liší (FORDE ET LORENZO 2001), např. trávy reagují na nedostatek vody podstatně rychleji (nárůstem kořenové biomasy) než leguminózy (SKINNER ET COMAS 2010).

Symbiotický vztah s bakteriemi rodu *Rhizobium* s. l.

Čeleď *Fabaceae* je známá svým symbiotickým vztahem s bakteriemi rodu *Rhizobium* s. l., které jsou schopny poutat N_2, což je u této čeledi významnou konkurenční výhodou v prostředí s nedostatkem NO_3^- a NH_4^+ (HONSOVÁ ET AL. 2007, JACKSON ET AL. 2008). Biologická fixace dusíku je energeticky náročný děj a je specifická pouze pro určitý úzký okruh organismů, u kterých je přítomný enzymový komplex nitrogenáza.

Množství leguminózami fixovaného dusíku dosahuje běžně hodnot 200 až 300 kg N ha^{-1} rok^{-1}. Např. druh *M. sativa* je schopný fixovat až 350 kg N ha^{-1} rok^{-1}, *T. pratense* 375 kg N ha^{-1} rok^{-1} v závislosti na faktorech prostředí (ZAHRAN 1999, CARLSSON ET HUSS-DANELL 2003). Výnos plodin v osevním postupu po leguminózách je porovnatelný s aplikací minerálního N hnojiva o dávce 30 - 80 kg N ha^{-1} (PEOPLES ET AL. 1995). Bakterií rodu *Rhizobium* s. l. se dnes užívá také k inokulaci půdy nebo semen rostlin čeledi *Fabaceae*. Očkováním osiva leguminóz lze zvýšit výnos plodin minimálně o 5 % v závislosti na podmínkách prostředí (NĚMEC 1986).

Nodulace a její průběh

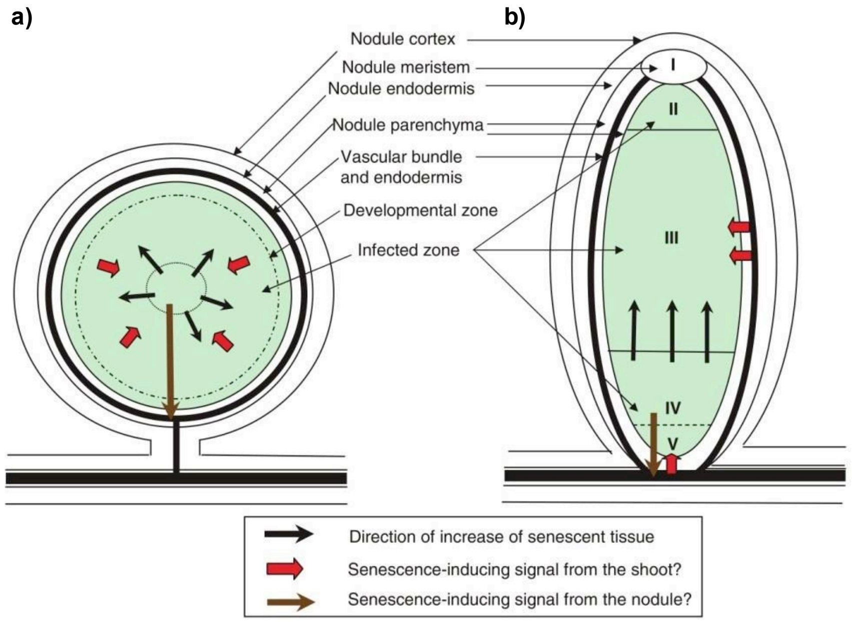
Nodulace je podobně jako kořenový systém ovlivněna podmínkami prostředí (ZAHRAN 1999), rostlinou (jejími chemickými, fyzikálními a genetickými vlastnostmi) a přítomností bakterií v půdě (WILLEMS 2006). Hlízkovité bakterie jsou součástí mikrobiálního společenstva v půdě. Volně žijící bakterie upřednostňují saprotrofický způsob života, přičemž většinou dusík nefixují (GADE 2004).

Kořeny rostlin čeledi *Fabaceae* vylučují do půdy flavonoidy, např. luteolin, betainy nebo kyselinu aldonovou (BEGUM ET AL. 2001). V nízkých koncentracích tyto látky působí jako signál pro chemotaxi bakterie. Ve vyšších koncentracích při povrchu kořene spouští expresi plazmidových nod-genů pro tvorbu lipochitooligo-sacharidových molekul tzv. nod-faktorů (CATOIRA ET AL. 2000). Rostliny produkují druhově specifickou směs flavonoidů, která umožňuje přijetí tohoto signálu vhodným druhem bakterie (STOCHMAL ET OLESZEK 2007). Bakterie na ně reaguje a infikuje se do kořene kořenovými vlásky (MÖLLEROVÁ 2006). Rostlina zároveň utváří na povrchu svých kořenových vlásků lektiny, které usnadňují průnik bakterie do vlásku (PARNISKE 2004).

Hlízky

Místo vzniku hlízek se nachází většinou za kořenovou špičkou (Kutschera et Lichtenegger 2002). Většina hlízek se nachází na tenkých laterálních kořenech, které jsou náchylnější k infekci bakteriemi (Capoen et al. 2010).

Leguminózy utvářejí hlízky dvojího typu determinované a nedeterminované (Obr. 2). Příkladem leguminózy s determinovanými hlízkami je *Lotus japonicus*. Nedeterminované hlízky nalézáme u jiné modelové leguminózy *Medicago truncatula* (Oldroyd et al. 2011). U nedeterminovaných hlízek dochází k dělení v apikální části, v bazální části dochází ke stárnutí a odumírání (Puppo et al. 2005). Oproti tomu determinované hlízky se vyvíjí jako celek (Gonzalez-Rizzo et al. 2009). Determinované hlízky se zakládají ve vnějších vrstvách primární kůry. Meristém hlízek tohoto typu se brzy diferencuje a přestává se dělit, což vede k jejich malé velikosti a kulovitému tvaru (Diouf et al. 2003).



Obr. 2: a) Determinovaná a b) nedeterminovaná hlízka (Puppo et al. 2005).

Tvar hlízek je ovlivněn především taxonomií rostliny a symbiotickými bakteriemi. Corby (1988) poukazuje na vliv tribové příslušnost rostliny, Zahran (1998) a Rejili et al. (2007) na jejich druhovou specifičnost. Hlízky mohou být například kulaté, zploštělé, polokulovité, podlouhlé, korálovité nebo větvené (Corby 1971, Möllerová 1978). Dokonce i samotné zduření kořene u leguminóz lze považovat za hlízku (Sprent et al. 2013).

Hlízky mohou dosáhnout až osminásobku průměru kořenu. Mohou zůstat zcela nevětvené nebo se i mnohonásobně větvit (KUTSCHERA ET LICHTENEGGER 2002). Hlízky některých druhů rostlin mohou dokonce přezimovat, což se projevuje jejich zúžením (PATE 1958A).

U nedeterminovaných hlízek dochází k postupné změně barvy během vegetačního období. Mladé hlízky jsou bílé a drobné, starší mají narůžovělou barvu (obsahují leghemoglobin a bakteroidy), což vypovídá o aktivní fixaci dusíku (PUPPO ET AL. 2005). Stárnutí hlízek probíhá od kořene (od báze hlízky k jejímu vrcholu), kdy dochází k rozkladu leghemoglobinu a hlízka získává nazelenalou barvu. Stárnoucí a odumírající hlízky jsou pak hnědé (PATE 1958B).

Velikost hlízek se mění v průběhu vegetační fáze rostliny, hlízky se vyvíjí do té doby, kdy se začínají vyvíjet semena.

Mykorhiza

U rostlin čeledi *Fabaceae* lze také vysledovat endotrofní vezikulární-arbuskulární mykorhizu. Houby řádu *Glomales* (třída *Zygomycetes*) mohou reagovat na přítomnost hlízek (ROSENDAHL ET DODD 1995, PASZKOWSKI 2006). Mykorrhizní houby nefixují atmosférický dusík, ale díky fosforu poskytující rostlině podporují aktivitu nitrogenázy (PUPPI 1983, RABIE ET AL-HUMIANY 2004). U rostlin je možné najít i velmi složitý symbiotický vztah např. rostlina - *Rhizobium* - houba. V tomto vztahu jsou obsaženy dva mutualistické vztahy, které se vzájemně doplňují (DIOUF ET AL. 2003). Mykorhiza prospívá rostlinám hlavně prostřednictvím fosforu, ale i dusíku, draslíku, a zinku (ALLEN ET ALLEN 1981, SMITH ET READ 1997). Mykorhiza nebyla objektem výzkum této práce a její přítomnost nebyla nijak zohledněna.

Zástupci čeledi *Fabaceae*

Medicago sativa L., tolice setá

Druh *M. sativa* je hemikryptofyt pocházející ze střední Asie (KUTSCHERA 1960). Jedná se o jednu ze světově nejvýznamnějších pícnin. Má vysokou krmivářskou hodnotu a vysoký obsah proteinů (SLAVÍK 1995).

Obr. 3: Nadzemní a podzemní část druhu *M. sativa* (CHMELÍKOVÁ 2009).

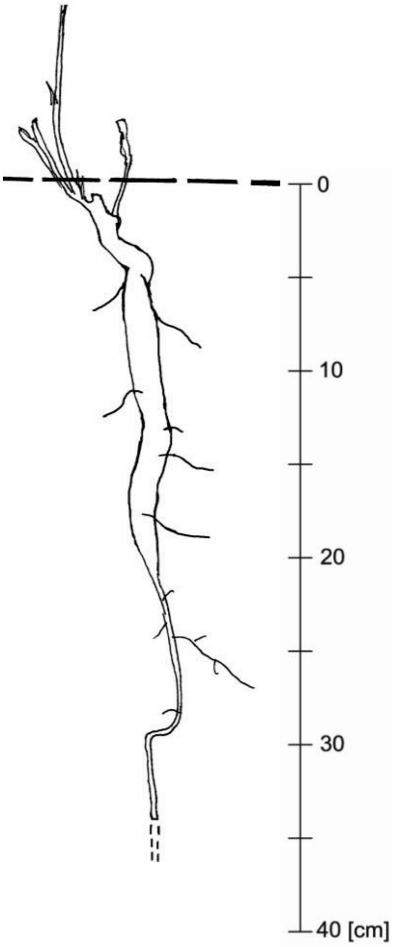
Ekologie druhu

Druh *M. sativa* je pěstován jako pícnina, která často zplaňuje. Šíří se do původních společenstev, ale diversitu nesnižuje (MLÍKOVSKÝ ET STÝBLO 2006). Najdeme jej na okrajích cest a náspech. S oblibou roste na sušších, zvláště ruderalizovaných loukách, okrajích polí, rumištích apod. Roste spíše na výhřevných, provzdušněných, živinami bohatých půdách, jejichž pH je často neutrální, někdy dokonce mírně kyselé či mírně zásadité (PETERS ET AL. 2005). Dobře odolává suchu a je vápnomilná (VOLF 1988).

Vyskytuje se po celém území od nížin do podhůří, ve společenstvech svazů *Dauco - Melilotion* Görs 1966, *Convolvulo - Agropyrion* Görs 1966, *Bromion erecti* Koch 1926 a *Arrhenatherion* Koch 1926 (MORAVEC1995, SLAVÍK 1995). Díky svému kontinentálnímu původu a hluboko sahajícím kořenům je tento druh odolný vůči mrazu a suchu (KUTSCHERA 1960, ELLENBERG ET AL. 1992). Životní strategie *M. sativa* (*sensu* GRIME 2001) je konkurenční stratég (KLOTZ ET AL. 2002), přesněji C/CSR (HODGSON ET AL. 1999).

Kořenový systém

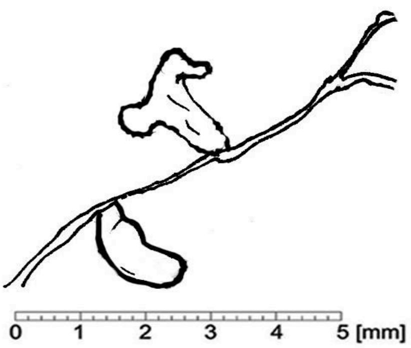
Mladé rostliny mají bohatě větvený kořenový systém až do 5. řádu, starší rostliny jsou oproti nim charakteristické svým kůlovým kořenem směřující kolmo dolů (CHMELÍKOVÁ 2009, KUTSCHERA ET AL. 2009). Uvádí se, že je kořen schopen růst až do hloubky několika metrů (FRANKOW-LINDBERG ET DAHLIN 2013). Tento silný kořen se většinou téměř nevětví, při povrchu utváří jen málo kratších postranních kořenů (KUTSCHERA 1960). Ve větších hloubkách je více členěn a je schopen utvářet i silnější postranní kořeny (HAKL ET AL. 2012A). Kořen má barvu světle žlutohnědou až tmavě hnědou (KUTSCHERA 1960, CHMELÍKOVÁ 2009). Obvod kořene v nejširších místech (několik cm pod zemí) činí až několik centimetrů (CHMELÍKOVÁ 2009). Ačkoliv rostlina upřednostňuje dostatek vzduchu v půdě, kořeny dorůstají do velkých hloubek i v půdách s nízkým obsahem kyslíku (KUTSCHERA ET AL. 2009, CHMELÍKOVÁ ET AL. SUBMITTED A).



Obr. 4: Kořenový systém *M. sativa* (CHMELÍKOVÁ 2009).

Hlízky

Hlízky se obvykle utváří na jemných kořenech, což vysvětluje absenci hlízek u starších rostlin. U mladších rostlin se utváří na kořenech 1. a 2. řádu (CHMELÍKOVÁ 2009). Jejich tvar bývá kulatý, válcovitý i větvený (MARX 2004). Válcovité hlízky dosahují nejčastěji rozměrů 1 x 2 mm, kulaté až 2 mm a větvené až 3 x 3 mm.



Obr. 5: Hlízka *M. sativa* (CHMELÍKOVÁ 2009).

Trifolium arvense L., jetel rolní

Tento druh pochází z Evropy a východní Asie (KUTSCHERA 1960). *T. arvense*, známý též jako jetel zaječí, se používá často v lidovém léčitelství (AICHELE ET GOLTE-BECHTLE 2005). Jedná se o jednoletý až dvouletý terofyt (ROTHMALER ET AL. 2000, KLEYER ET AL. 2008).

Obr. 6: Podzemní i nadzemní část *T. arvense* (CHMELÍKOVÁ 2009).

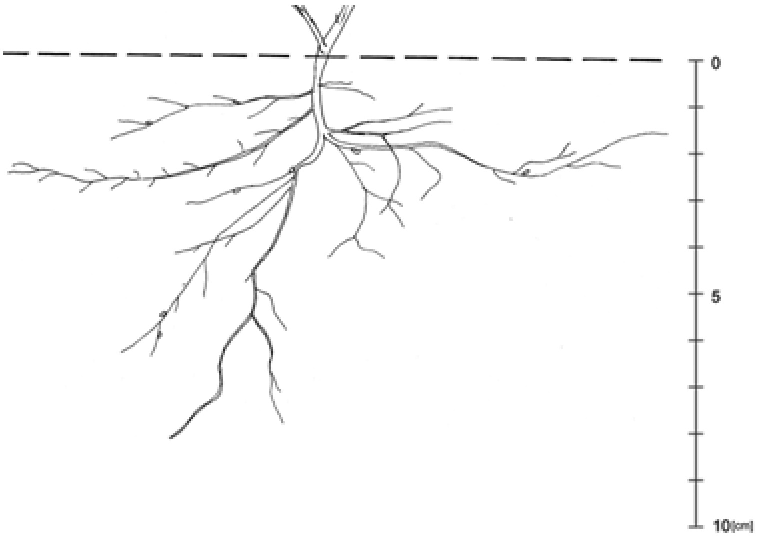
Ekologie druhu

Trifolium arvense roste na suchých slunných stanovištích. Vyskytuje se převážně na chudých nevápnitých, kyselých, mělkých dobře provzdušněných půdách s rozvolněnou vegetací (SLAVÍK 1995, SCHAUER 2007). Tento druh lze označit indikátorem kyselých půd.

Nalezneme ho od nížin do nižších poloh submontánního stupně, na celém území ČR roztroušeně až hojně, v oreofytiku vzácně. Je diagnostickým druhem svazu *Plantagini - Festucion ovinae* Passarge 1964 (MORAVEC 1995), často přesahující do dalších společenstev třídy *Sedo - Scleranthetea* Br. - Bl. 1955 em. Moravec 1967 (SLAVÍK 1995). Životní strategie *T. arvense* (*sensu* GRIME 2001) je stres tolerantní R-stratég, přesněji R/SR (HODGSON ET AL. 1999).

Kořenový systém

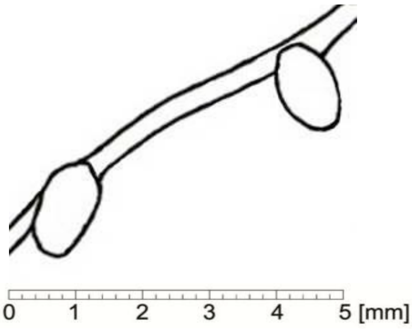
Tento druh má větvené kořenové systémy, u kterých je možné ve většině případů vysledovat hlavní kořen (CHMELÍKOVÁ 2009). Hlavní kořen směřuje kolmo dolů či se plazí těsně pod povrchem půdy, nejvýše však do hloubky 10 cm (FITTER ET PEAT 1994, CHMELÍKOVÁ 2009), ačkoliv KUTSCHERA (1960) zaznamenala kořeny až do hloubky 40 cm. Průměr kořenového krčku dosahuje maximálně 5 mm. Kořeny se větví do 2. řádu, vzácně do 3. řádu. Barva kořenového systému je bledavě hnědá. Kořeny vyšších řádů jsou často tmavší než hlavní kořen.



Obr. 7: Kořenový systém *T. arvense* (CHMELÍKOVÁ 2009).

Hlízky

Hlízky se u tohoto druhu nachází na kořenech 1. a 2. řádu, někdy dokonce i na hlavním kořeni (CHMELÍKOVÁ ET HEJCMAN 2012b). Jejich tvar je většinou válcovitý, někdy kulatý, výjimečně lze pozorovat i hlízky prstovitě větvené. Hlízky obvykle dosahují jen malých rozměrů - 1 - 2 mm (CHMELÍKOVÁ 2009).



Obr. 8: Hlízky *T. arvense* (CHMELÍKOVÁ 2009).

Trifolium medium L., jetel prostřední

Tento hemikryptofyt s načervenalou poléhavou nebo častěji vystoupavou lodyhou, která se bohatě větví, je původem z Evropy a západní Asie (FITTER ET PEAT 1994). Jedná se o pícninářskou rostlinu. Výzkumy ukazují, že je ve srovnání s *T. pratense* odolnější proti poškození podzemích částí těžkou mechanizací, a že je mimořádně rezistentní proti virovým chorobám (SLAVÍK 1995).

Obr. 9: Podzemní i nadzemní část *T. medium* (CHMELÍKOVÁ 2009).

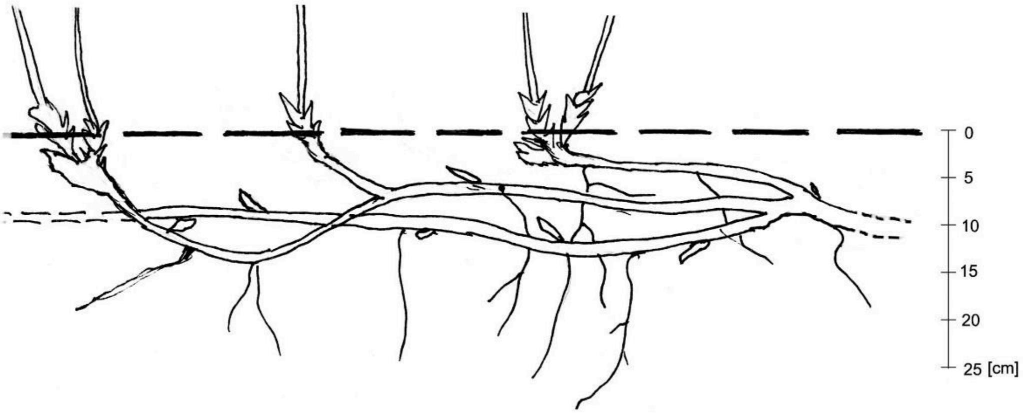
Ekologie druhu

Trifolium medium je ekotonní (ekotonální) druh, roste na okrajích lesů, křovinatých i travnatých strání, na loukách a pastvinách. Nalezneme ho na mírně suchých až čerstvě vlhkých, kyprých a částečně zastíněných místech, a to jak na kyselých, tak i na alkalických substrátech (ELLENBERG ET AL. 1992, AICHELE ET GOLTE-BECHTLE 2005).

Tento druh je u nás rozšířen hojně od nižších pahorkatin do hor, optimum výskytu je v mezofytiku a v přilehlých územích termofytika a oreofytika, vzácně chybí v teplejších a sušších oblastech a v horách nad 800 m. n. m. (SLAVÍK 1995). Je diagnostickým druhem svazu *Trifolion medii* Th. (MÜLLER 1962), častý je též ve společenstvech řádu *Prunetalia* Tüxen 1952 (MORAVEC 1995). Životní strategie *T. medium* (*sensu* GRIME 2001) je stres tolerantní konkurenční stratég, přesněji SC/CSR (HODGSON ET AL. 1999).

Kořenový systém

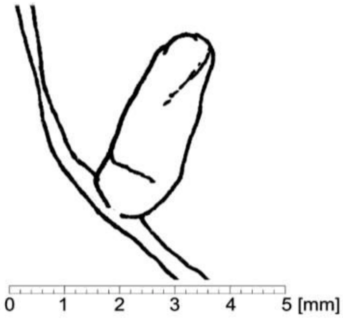
Trifolium medium utváří rozsáhlý kořenový systém s mělkými plazivými oddenky (FITTER ET PEAT 1994, KLIMEŠOVÁ ET DE BELLO 2009). Mimo oddenků utváří tento druh také hlavní kůlový kořen. Největšího průměru kořene dosahuje rostlina v hloubce několika centimetrů pod povrchem půdy. Kořeny se větví maximálně do 3. řádu. Barva silnějších kořenů je žlutohnědá, některé kořeny 1. řádu mohou být světlé až bílé, kořeny vyšších řádu mohou být tmavší (CHMELÍKOVÁ 2009).



Obr. 10: Kořenový systém *T. medium* (CHMELÍKOVÁ 2009).

Hlízky

Hlízky u tohoto druhu preferují, stejně jako u většiny ostatních druhů, jemné kořeny 2. a 3. řádů. Druh *T. medium* utváří podobně jako všechny rostliny druhy rodu *Trifolium* spp. především válcovité hlízky, lze však nalézt i hlízky kulatého, kyjovitého a vzácně větveného tvaru (CHMELÍKOVÁ ET HEJCMAN 2013). Jejich rozměry se pohybují mezi 1 x 2 mm až 2 x 5 mm (CHMELÍKOVÁ 2009).



Obr. 11: Hlízka *T. medium* (CHMELÍKOVÁ 2009).

Trifolium pratense L., jetel luční

Tento hemikryptofyt je dvouletá až víceletá bylina s četnými lodyhami původem z Evropy a přilehlých částí Asie a Afriky (SLAVÍK 1995). Je důležitou pícninou bohatou na bílkoviny (AICHELE ET GOLTE-BECHTLE 2005). Druh *T. pratense* je pěstován také jako alternativní druh na místech nevhodných pro *M. sativa*. Z důvodu zachování výnosu i v dalším roce je častěji pěstován ve směskách než v monokultuře (HALLING ET AL. 2002).

Obr. 12: Podzemní i nadzemní část *T. pratense* (CHMELÍKOVÁ 2009).

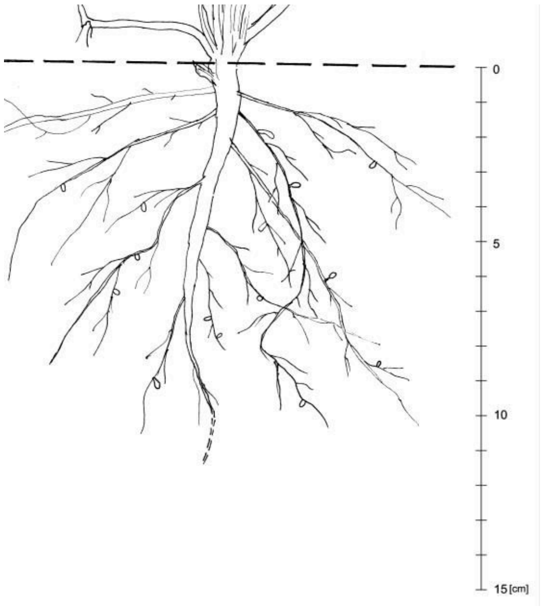
Ekologie druhu

Trifolium pratense je druh suchých až mírně vlhkých luk, často je pěstován na polích v jetelotravních směskách. Roste na pastvinách a na okrajích cest, v oblibě má výživné hluboké půdy (AICHELE ET GOLTE-BECHTLE 2005, SCHAUER 2007).

Na našem území ho nalezneme nejen na mezofilních loukách nížin, ale i v oblastech podhorského stupně (vzácně i na horách). Je diagnostickým druhem svazu *Arrhenatherion* Koch 1926 (MORAVEC 1995). Oproti *M. sativa* je méně odolný vůči suchu a mrazu, na druhou stranu je však odolnější vůči zamokření (KUTSCHERA 1960). Životní strategie *T. pratense* (*sensu* GRIME 2001) je konkurenční stratég (KLOTZ ET AL. 2002), přesněji CSR (HODGSON ET AL. 1999).

Kořenový systém

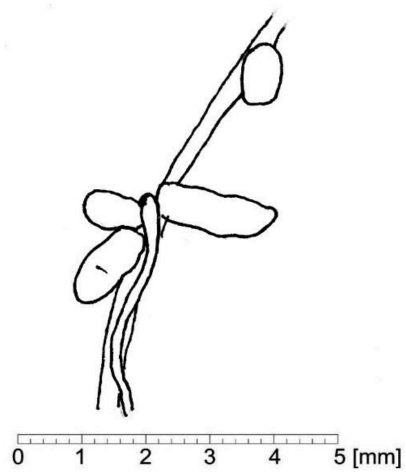
Rostliny tohoto druhu mají kořenový systém se zřetelným hlavním kořenem, který se bohatě větví až do 5. řádu (KUTSCHERA 1960). Laterální kořeny bývají často velmi dlouhé, až do vzdálenosti 20 cm od rostliny (CHMELÍKOVÁ 2009). Starší rostliny mohou v kořenovém krčku dosahovat až několika cm. Oproti *M. sativa* kořeny nedosahují takových hloubek (KUTSCHERA 1960). Barva kořene je světle hnědá (někdy lehce narůžovělé), žlutavá až bělavá. Po disturbanci (nalomení lodyhy) byla zjištěna schopnost utváření adventivních kořenů na lodyze (CHMELÍKOVÁ 2009).



Obr. 13: Kořenový systém *T. pratense* (CHMELÍKOVÁ 2009).

Hlízky

Tento druh utváří velké množství hlízek nezávisle na stanovišti (CHMELÍKOVÁ ET AL. SUBMITTED B). Jemné laterální kořeny k nodulaci přímo vybízejí. Jako druh rodu *Trifolium* spp. nese na svých kořenech hlízky tvaru válcovitého, někdy i kulatého a kyjovitého tvaru. Rozměry hlízek dosahují většinou 1 x 2 mm až 1 x 3 mm.



Obr. 14: Hlízky *T. pratense* (CHMELÍKOVÁ 2009).

Zvolené faktory ovlivňující morfologii kořenových systémů a nodulaci

Životní forma rostlin (Raunkiaer 1934)

Kořeny jednoletých a víceletých rostlin se liší svoji stavbou a velikostí (Fitter et al. 1988, Gross et al. 1992, Roumet et al. 2008). Víceleté rostliny utvářejí více větvený kořenový systém s větším průměrem. Dichotomicky větvené kořenové systémy jsou typické pro jednoleté rostliny (Gross et al. 1993). Větvení ovlivňuje strategii příjmu živin (Roumet et al. 2006).

Mnoho rostlin má nezávisle na jejich příslušnosti k taxonomickému systému v hrubých rysech podobný vzhled (Fischer 2003), tento vzhled odpovídá životním formám dle Raunkiaera (1934), často jsou tyto formy vzhledu bez zohlednění míry ekologického přizpůsobení označovány jako formy růstu (Fischer 2003).

Klasifikace životních forem dle Raunkiaera (1934) vychází z pozice, ve které rostlina vytváří a zachovává obnovovací pupeny během nepříznivého období (Kovář 2002). Tato klasifikace byla vícekrát přepracována a zjemněna a dnes je možné všechny vyšší rostliny zařadit do devíti hlavních skupin. Tyto hlavní skupiny lze ještě dále členit a specifikovat (Müller-Dombois et Ellenberg 1974), aby se zdůraznila morfologická přizpůsobení na zvláštní podmínky (Fischer 2003). Mezi hlavní životní formy, které jsou zastoupeny v předložené práci, patří fanerofyty, hemikryptofyty, chamaefyty a terofyty. Dalšími formami jsou geofyty, liány a epifyty, parazité a hydrofyty (Fischer 2003, Knevel et al. 2005).

Hemikryptofyty ukrývají své obnovovací pupeny při povrchu půdy a jsou chráněny vrstvou opadanky. Terofyty se rychle vyvíjí na počátku vegetačního období a tvoří v krátké době mnoho odolných semen, načež rostlina sama odumře. Chamaefyty utváří zdřevnatělé stonky a větve, na kterých se nachází orgány pro přečkání nepříznivých období, dosahují nejvýše 50 cm nad zemský povrch (nízké keře). Obnovovací orgány fanerofytů se nachází více než 50 cm nad zemí na zdřevnatělých a dlouhověkých větvích nesoucích pupeny.

Dostupnost živin

Dostupnost živin je složité přesně definovat a ještě složitější je dostupné živiny přesně určit. Dostupnost živin je totiž závislá na mnoha faktorech, které není možné předem určit. Příkladem je růst kořene, vlhkost půdy a činnost mikroorganismů (MENGEL ET AL. 2001). Příjem živin je regulován růstem a metabolismem rostliny a koncentrací živin v půdě (AMBERGER 1983). Rostlina má schopnost zvýšit příjem živin tím, že svými kořeny produkuje do prostředí exudáty. Tyto exudáty spolu s odumřelými vlásky a slizem podporují aktivitu mikroorganismů, které na oplátku podporují dostupnost živin (MARSCHNER 1995).

Rostliny jsou na dostupnosti živin v půdě životně závislé. Jak udává Sprengel-Liebigův zákon minima (LIEBIG 1840, LIEBIG 1855, PLOEG ET AL. 1999) je pro růst rostlin limitující právě ten prvek, který je v minimu. Nároky rostlin na jednotlivé živiny jsou tak ovlivněny dostupností ostatních živin (TRUONGT ET BRIX 2009). Ve většině rostlinných společenstev se jedná o dusík, fosfor a draslík. Vzájemný poměr dostupnosti jednotlivých živin v půdě hraje důležitou roli. Také může docházet i k limitaci dvou živin současně (GÜSEWELL 2004, KNECHT ET GÖRANSSON 2004, AGREN ET AL. 2012). Nároky rostlin na živiny jsou u jednotlivých druhů rostlin značně rozdílné.

Většina studií je zaměřena především na dostupnost tří základních živiny - NPK. Několik prací však poukazuje na významný vliv i jiných živin (i na kořenové systémy), jako například na síru (BOSWELL ET AL. 2007, SOUSSANA ET TALLEC 2009). Dusík, fosfor a draslík mají zásadní význam pro růst rostlin. Studium adaptace rostlin na různou dostupnost těchto živin je tedy velmi aktuálním tématem. Převážná část prací se však zaměřuje především na vliv na výnos nadzemních částí rostlin (HEJCMAN ET AL. 2010) a to hlavně zemědělských plodin (HENRY ET CHINEDU 2014, IZSAKI 2014, KUMAR ET BOHRA 2014, SATTARI ET AL. 2014).

Rostlina přijímá podstatnou většinu živin kořeny. Živiny se dostávají do kořene intercepcí, pomocí transpiračního hmotového toku a difúzí (MARSCHNER 1995, PLHÁK 2003). Kořeny rostlin přijímají z půdy živiny ve formě kationtů (např. K^+, NH_4^+, Ca_2^+) a aniontů (např. NO_3^-, SO_4^{2-}, PO_4^{3-}). Příjem živin je ovlivněn jak samotnou rostlinou, tak podmínkami prostředí (TRÁVNÍK 2010). Mezi podmínky prostředí patří povětrnostní podmínky (teplota, množství a rozdělení srážek) a především vlastnosti půdy, např. zrnitost, vlhkost, pórovitost, pH, obsah živin aj. (BAEUMER 1992, VOPRAVIL ET KHEL 2010). Např. MENDOZA ET AL. (2005)

zaznamenal u *Lotus glaber* ve vlhkých půdách vyšší obsah N a P v kořenech a v celé rostlině než u rostlin na suchých půdách. Stavba kořenového systému je jedním z nejdůležitějších parametrů rostliny, jež se projevuje v příjmu živin.

Plasticita rostlin umožňuje lepší zachycení mobilních iontů - jako jsou NO_3^- ionty, jejichž příjem není omezen difúzí v půdě, ale zaváděním k povrchu kořene. Pravý opak platí u imobilních iontů - jako je např. PO_4^{3-} (HODGE 2004). Větší délka kořenů spolu s jejich hustotou umožňuje zachycení imobilních iontů. Roli zde hrají i mykorhizní houby. Podle HØGH-JENSEN ET PEDERSEN (2003) a WRAGE ET AL. (2010) jsou rostliny díky své plasticitě schopné navýšit počet kořenů v půdě s nedostatkem fosforu a draslíku, aby se zvětšil povrch kořenového systému a došlo tak k navýšení příjmu těchto dvou živin.

V rámci disertační práce je pozornost věnována dostupnosti dusíku, fosforu a draslíku. Pozornost je z části zaměřena také na molybden, jež má specifickou úlohu u rostlin čeledi *Fabaceae*.

Dusík

Dusík patří k základním stavebním prvkům, které tvoří bílkoviny. Rostliny mají podstatně větší potřebu dusíku než ostatních živin (AMBERGER 1983). Je obsažen v rostlinných a živočišných reziduích, humusových látkách vznikající z organických látek aj. Dusík v této formě prochází procesem mineralizace, mění se na amoniakální (NH^{4+}) a dále na nitrátovou formu (NO^{3-}) a právě v těchto formách je dusík pro rostliny přijatelný.

O příjmu výše uvedených iontů rozhodují vnější podmínky, ale i rostlina sama (TESAŘ ET AL. 1992). Regulace příjmu dusíku ve formě NO_3^- je řízena celou rostlinou, konkrétně stavem dusíku v celé rostlině. Oproti tomu příjem dusíku ve formě NH_4^+ je především pod kontrolou kořenů a je lépe přijímán než NO_3^- (JACKSON ET AL. 2008). Velký vliv má pH prostředí, v kyselých půdách převažuje příjem NO_3^-. Na neutrálních až alkalických půdách se pak příjem obou iontů vyrovnává nebo převažuje příjem NH_4^+ (AMBERGER 1983). V lehkých písčitých půdách je dusíku méně, v těžkých půdách je dusík zastoupen ve velkém množství (MENGEL ET AL. 2001).

Rostliny čeledi *Fabaceae* díky svým symbiotickým bakteriím jsou schopné využívat také vzdušný dusík (N_2). Tyto rostliny jsou velmi citlivé na nadbytek N v raných fázích, tedy již při vzcházení (ŠNOBL ET AL. 2005). Velké množství minerálního dusíku snižuje, příp. inhibuje nodulaci (HIRSCH 1992, ROUMET ET AL. 2008).

Fosfor

Fosfor je živinou urychlující zrání, podporuje nasazení květů a zároveň přispívá k tvorbě pevných pletiv. Je nutný pro přenos dědičných vlastností, energie ATP a ADP, dělení buněk a fotosyntézu (TESAŘ ET AL 1992). V půdě je fosfor přítomný v organické a minerální formě. Organický fosfor je převážně obsažen ve fosfolipidech, nukleových kyselinách a inositolfosfátech (VANĚK 2007). Minerální fosfor je součástí anorganických sloučenin, ve kterých je ortofosforečnanový aniont ($H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}) vázán především na železo (Fe) a hliník (Al) v půdách kyselých a na vápník v půdách alkalických. Vyšší obsah fosforu vykazují půdy s větším obsahem organické hmoty (ŠNOBL ET AL. 2005). Převážná část fosforu je pro rostliny nepřijatelná. Organický fosfor v podobě odumřelých organismů je nedílnou součástí organické půdní hmoty (ŠNOBL ET AL. 2005). Fosfor je přijímán rostlinami ve formě aniontů $H_2PO_4^-$ a HPO_4^{2-}. Rostliny jsou schopné přijímat fosfor i při velmi nízké koncentraci v půdním roztoku, tento příjem je však energeticky náročný (TESAŘ ET AL. 1992). Nároky jednotlivých druhů na fosfor se výrazně liší.

U leguminóz, které nejsou limitovány dusíkem v půdě, lze fosfor označit za nejvýznamnější a zároveň limitující živinu. Fosfor ovlivňuje jak morfologii kořenových systémů, tak nodulaci (ADAMS ET AL. 2002). Pozitivní vliv fosforu byl zaznamenán na průměr kořene *M. sativa* a *Lotus australis* (PATREZE ET CORDEIRO 2005, PANG ET AL. 2010). Oproti tomu na půdách s nedostatkem fosforu se utvářely mělké kořeny s bohatším větvením (LYNCH 1995).

Rostliny se adaptují na příjem fosforu utvářením bohatšího kořenového systému, vylučováním více kořenových výměšků či využíváním symbiózy s houbami (WRAGE ET AL. 2010). Např. rod *Lupinus* spp. má schopnost osvojovat si živiny (zejm. fosfor) i ze špatně dostupných forem pomocí utváření krátkověkých kořínků, tzv. cluster roots (SHANE ET AL. 2008).

Draslík

Draslík je přijímán rostlinami jako kationt K^+. Příjem kationtu K^+ je buď aktivní, převládá při nižších koncentracích draslíku v půdě, nebo pasivní, který probíhá při jeho vysokých koncentracích (ŠNOBL ET AL. 2005). Draslík významně ovlivňuje aktivitu enzymů, a tak zasahuje do celé řady metabolických procesů, např. fotosyntézy a dýchání (VANĚK 2007). Draslík také zlepšuje odolnost rostlin vůči prostředí (ZAHRAN 1999).

Důležitým faktorem ovlivňujícím příjem draslíku rostlinou je mimo jiné zrnitost půdy. Rostliny odčerpávají draslík snadněji na lehčích písčitých půdách s nižší sorpční kapacitou (ŠNOBL ET AL. 2005). Obsah K^+ v kořenu je důležitý pro příjem vody a vznik kořenového vztlaku.

V rostlině je draslík velmi pohyblivý, z kořenů do prýtu je K^+ transportován xylémem, ze starších částí rostliny do částí mladých floémem. Leguminózy ukládají ve své biomase méně draslíku než jednoděložné rostliny, což naznačuje větší efektivitu hospodaření s draslíkem (HØGH-JENSEN ET PEDERSEN 2003).

Molybden

Rostliny přijímají molybden převážně jako aniont, ve formě MoO_4^{2-} (XU ET AL. 2013). Jeho potřeba je všeobecně pro většinu rostlin velmi nízká. Relativně nejvíce molybdenu vyžadují rostliny *Fabaceae*, příp. bakterie na kořenech těchto rostlin.

Molybden je prvek nezastupitelný v metabolizmu dusíku a síry. Je složkou enzymu nitrogenázy, kterým prokaryotické organizmy žijící v symbióze s vyššími rostlinami fixují vzdušný N_2 (YANG ET AL. 2014). Aktuální potřeba Mo v rostlině závisí na formě přijímaného dusíku. Převládá-li ve výživě NO_3^-, stoupá také potřeba Mo (ROMERO ET AL. 2013).

Dobrá dostupnost molybdenu v půdě pozitivně ovlivňuje nodulaci, např. množství a velikost hlízek (TOLEDO ET AL. 2010). Dobrá dostupnost molybdenu se projevuje prodloužení aktivity hlízek (SHAW ET AL. 1966).

Půdní reakce

Dostupnost živin závisí mimo jiné na hodnotě půdní reakce. Rostliny jsou schopné přijímat určité živiny jen v určitém rozmezí reakce půdy. Shelfordův zákon tolerance uvádí, že rostlina toleruje pouze určité rozpětí hodnot prostředí (např. pH), ve kterém je schopná růst a vyvíjet se.

Hodnota pH půdy je pro řadu druhů rostlin limitujícím faktorem, příkladem toho je *M. sativa* v Austrálii (Bettenay et Hingston 1964). Půdní reakce ovlivňuje klíčivost, vzcházivosti a růst rostlin (Mandák et Pyšek 2001, Křišťálová et al. 2011). Hodnoty pH prostředí limitují také růst kořenových systémů leguminóz a nodulaci (Panciera et Sparrow 1995, Brauer et al. 2002). Půdní reakce se projevuje v dostupnosti živin a příjmu živin rostlinou (Cruz 1997, Peters et al. 2005).

Rostliny čeledi *Fabaceae* preferují především slabě kyselé půdy (Bordeleau et Prevost 1994, Chmelíková et Hejcman 2012). Pro většinu druhů této čeledi je uváděno, že půdy s hodnotami pH nižší než 4 a vyšší než 8 jsou pro ně toxické (Bordeleau et Prevost 1994). Na těchto půdách dochází k nízké mobilitě fosforu (Baligar 1987), který je jednou z nejdůležitějších živin právě u leguminóz (Dodd et Orr 1995). Jednotlivé druhy rostlin reagují na pH půdy rozdílně citlivě. Např. podle Rice et al. (1977) se rozdílné hodnoty pH půdy (4,5 - 7,2) neprojevují ve výnosu a nodulaci *T. pratense*. Oproti tomu *M. sativa* dosahuje dobrých výnosů jen za určitých hodnot pH. Jako ideální se pro tento druh uvádí neutrální až mírně zásadité pH půdy (Rhykerd et Overdahl 1972), přesněji pH v rozmezí 6,5 - 7,0 (Peters et al. 2005).

Vliv půdní reakce se projevuje i při nodulaci. Kořenové vlásky, které jsou pro nodulaci důležité, reagují citlivě na kyselost půd (Munns 1968). Nodulace závisí také na citlivosti bakterií *Rhizobium* s. l. vůči pH prostředí (Howieson et al. 1988). Vliv půdní reakce se projevuje např. tvorbou menších hlízek u *M. sativa* v kyselých půdách a tvorbou větších hlízek téhož druhu na neutrálních půdách (Cheng et al. 2002).

Samy kořeny leguminóz svými exudáty snižují pH v rhizosféře (Yan et al. 1996, Tang et al. 1999).

Půdní zrnitost (textura)

Zrnitost půdy ovlivňuje stavbu kořenového systému a jeho penetraci (pronikání) půdou. Podle Šmilauerové (2001) zlepšuje vyšší obsah písku v půdě schopnost penetrace kořene a podporuje jeho rovnoměrné větvení. Naopak ke zhoršení penetrace dochází při vyšším obsahu štěrku v půdě, kdy se kořeny větví a zvětšují svůj průměr v místech s možností snazšího průniku. Rozdílná penetrace kořene ovlivňuje příjem vody a živin rostlinou (Šmilauerová 2001) a následkem toho může dojít i ke snížení výnosu nadzemní biomasy (Salako

ET AL. 2002). Reakce rostlin na půdní zrnitost se mezi jednotlivými druhy liší (MIA ET AL. 1996, ZAHRAN 1998, FORDE ET LORENZO 2001, REJILI ET AL. 2007). Např. u *Plantago lanceolata* se více projevily rozdíly obsahu živin v půdě než heterogenita substrátu (ŠMILAUEROVÁ ET ŠMILAUER 2002).

Půdní zrnitost je ovlivněna zhutněním půdy (HAKL ET AL. 2007). Vliv zhutnění snižuje hustotu kořenových délek až o 60 % (GRIMES 1978). Následkem toho klesá také výnos plodin o 10 % (MEEK ET AL. 1988, RECHEL ET AL. 1990). Při zhutnění půdy dochází nejen ke změně zrnitosti půdy, ale zároveň ke změně její kompaktnosti, dostupnosti živin a schopnosti půdy zadržovat vodu (ANNICCHIARICO 2007), což ovlivňuje dále morfologii kořenových systémů a jejich prostorové uspořádání. Tento vliv se nejvíce projevuje ve svrchních 10 cm půdy, jak uvádějí KRISTOFFERSEN ET RILEY (2005) a GLAB (2008) pro kořeny *M. sativa*.

3. VÝSLEDKY DISERTAČNÍ PRÁCE

Článek I: Root system variability in common legumes in Central Europe

Článek II: Effect of nitrogen, phosphorus and potassium availability on emergence, nodulation and growth of acidicole *Trifolium arvense* L. in alkaline soil

Článek III: Effect of nitrogen, phosphorus and potassium availability on emergence, nodulation and growth of *Trifolium medium* L. in alkaline soil

Článek IV: Seasonal development of biomass yield in grass-legume mixtures on different soils and development of above- and below-ground organs of *Medicago sativa*

Článek V: Seasonal development of above- and below-ground organs of *Trifolium pratense* in grass-legume mixture on different soils

I. Root system variability in common legumes in Central Europe

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Root system variability in common legumes in Central Europe

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Abstract: The aim of this study was to provide an overview of field measured root systems of common legume species growing under different environmental conditions in the Czech Republic. The plants, 214 individuals of 21 selected legume species from the tribes *Galegeae* (*Astragalus glycyphyllos, Lupinus polyphyllus*), *Genisteae* (*Cytisus scoparius, Genista tinctoria*), *Loteae* (*Anthyllis vulneraria, Lotus corniculatus, Securigera varia*), *Trifolieae* (*Trifolium arvense, T. campestre, T. medium, T. pratense, T. repens*) and *Vicieae* (*Lathyrus pratensis, L. sylvestris, Vicia angustifolia, V. cracca, V. hirsuta*), were collected using the monolith method from 27 sites.
A rhizome was present in seven species and the maximum branching order was three for 15 species and five for five species. Recovery buds were recorded on the root system of eight species and woodiness was recorded in 11 species. Root diameter ranged from 1 to 12 mm – the minimum diameter was recorded in annuals and the maximum in perennials. The colour of the root system ranged from light to dark. In six species, young roots were light and older roots were dark. Globose, cylindrical, branched, fan-like and ruff-like nodules were recorded. Only one type of nodule shape was recorded in 11 species, two in seven species and three or four in three species. Nodules measured up to 2 mm in nine species, from 2 to 4 mm in three species and more than 4 mm in nine species. Legume root systems are highly variable and the variability was due to Raunkier's life forms rather than membership of a tribe.

Key words: annual and perennial species; environmental conditions; Fabaceae; nodules and nodulation; Raunkier's life form

Introduction

Legumes (family Fabaceae) comprise more than 650 genera and 18,000 species and are found almost worldwide (Doyle 2001). In Europe, the temperate herbaceous legumes are divided into eight tribes (Polhill 1981). Legumes are known for their symbiotic relationship with *Rhizobium* s.l. bacteria, enabling them to utilize aerial N_2, which is inaccessible to many other species (Garg & Geetanjali 2007; Novák 2010). Symbiotic nitrogen fixation makes legumes highly competitive especially in environments limited by mineral (NO_3^- and NH_4^+) nitrogen and with an adequate phosphorus and potassium supply (Honsová et al. 2007; Jackson et al. 2008). The presence of *Rhizobium* bacteria in the soil initiates nodulation – i.e. the creation of nodules of several shapes on the roots of legumes. Nodulation can be affected by soil salinity and pH, moisture, temperature and nutrient availability (Zahran 1999). In previous studies, the formation of nodules was recorded only when plants were grown in mineral nitrogen-deficient soils (Hirsch 1992), whereas high phosphorus together with high mineral nitrogen availability in the soil suppressed (Truongt & Brix 2009) or entirely inhibited nodulation (Roumet et al. 2008). According to Corby (1988), nodule shape is associated with legume taxonomy and can be species-specific (Zahran 1998) or tribe-specific (Rejili et al. 2007). In addition to nodulation, the response of the root system to soil conditions is believed to be life form (Johnson & Biondini 2001) or species-specific (Mia et al. 1996).

In this study, root systems were defined as the network of underground organs. As plants are sessile and therefore cannot select the best site for life, their root systems must be sufficiently plastic to cope with variable soil conditions, especially with water and nutrient availability (Briggs & Walters 1997; Hodge 2004; Sultan 2000; Šmilauerová & Šmilauer 2010).

The root systems of individual plants can be highly modified by root competition as the competition for nutrients provokes higher energy investment into roots in order to increase their density and length (Olde Venterink & Güsewell 2010). The presence of nutrient-rich patches in the soil profile may also modify the root system because roots are able to proliferate and grow preferentially into nutrient-rich patches (Robinson 1994; Forde & Lorenzo 2001; Hejcman et al. 2011).

Lucero et al. (1999) concluded that water deficit increases the rooting depth of *Trifolium repens.* According to Skinner & Comas (2010), drought stress increased not only the root depth of six legumes but also the length of their roots. On the other hand, waterlog-

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ging increased the root crown diameter of *Lotus glaber* in a study by Mendoza et al. (2005).

At the individual species level, some of the variability in root system morphology can be ascribed to life form and nutrient acquisition and conservation strategies. The root systems of perennials are more randomly branched and have larger diameter roots than annuals (Roumet et al. 2006, 2008). On the other hand, dichotomously branched root systems are more typical in annuals (Gross et al. 1993). This may explain the nutrient conservative strategy of perennials and the high nutrient uptake capacities of annuals recorded by Roumet et al. (2006). According to Antos & Halpern (1997), different annual species have a similar root morphology, differing from perennials especially in terms of rooting depth.

Root systems have been most frequently studied in grasses (Hodge et al. 1998; Janeček et al. 2007; Pecháčková et al. 2003; Šmilauerová & Šmilauer 2002; Van der Krift & Berendse 2002; Wahl et al. 2001; Wildová et al. 2007), field crops (Haberle & Svoboda 2000; Lamb et al. 2000; Merrill et al. 2002; Svoboda & Haberle 2006) and forage legumes (Braun et al. 2010; Hakl et al. 2007, 2011; Komárek et al. 2010), but there is a lack of detailed information about the root system morphology and variability of common legumes in Central Europe. Some results of plant excavations in the field were published by Kutschera (1960) and Kutschera & Lichtenegger (1992), but the root system variability of many common species such as *Vicia angustifolia*, *Vicia cracca*, *Lupinus polyphyllus*, *Lathyrus sylvestris*, *Astragalus glycyphyllos* and *Cytisus scoparius* has never been described.

The aim of this paper was therefore to provide an overview of field measured root systems of 21 common legume species growing under different environmental conditions in Central Europe. We asked the following research questions: (i) How does the morphology of the root systems plus nodules vary in the investigated species? (ii) Which root traits are characteristic for individual species?

Material and methods

Study area

The plants, 214 individuals of 21 selected legume species, were collected from 27 sites in the Czech Republic at an altitude ranging from 350 to 580 m. a.s.l. (see Table 1 for a list of investigated species and the number of sites from which each species was collected). The species belonged to five tribes (*Galegeae*, *Genisteae*, *Loteae*, *Trifolieae* and *Vicieae*). The names of the tribes follow Allen & Allen (1981). The average annual precipitation and temperature ranged from 600 mm and 8 °C at 350 m a.s.l. up to 700 mm and 6.5 °C at 580 m a.s.l. The sampling sites were described as dry or humid, field, forest, ruderal or meadow, sun exposed or shaded, stony or without stones in the upper 30 cm soil layer, and with low (up to 40%) or high (above 40%) cover of herbaceous vegetation in a 1-m diameter circle.

Plant collection

The plants were collected from June to August in the years 2007, 2008 and 2009. Collection of all species at all sampling sites was impossible as the investigated species had different environmental requirements, and therefore it was impossible to strictly separate the variability in root systems due to the species effect and that due to environmental conditions. The monolith method was used to collect individual plants (Böhm 1979). The soil monolith measuring 30 × 30 × 30 cm beneath the plant was dug up and washed with water to extract the underground organs of the studied plants from the soil. The plant species nomenclature follows Kubát et al. (2002).

Traits of root systems

The traits of root systems and nodules that were investigated are summarized in Table 1. Eight traits were evaluated for root systems: type of root system, presence of rhizomes, depth, special diameter, maximal branching order, colour and woodiness; and two traits for root nodules: shape and size. The type of root system was classified according to the database of clonal and bud bank traits of Central European flora (Klimešová & de Bello 2009). The life forms of the investigated species follow Raunkiaer (1934). The measured rooting depth was limited to 30 cm by the use of the monolith method. The special diameter is the mean value of taproot diameter under the crown and the maximum diameter within the root system. The root colour ranged from light to dark (light, near white, light/dark (young roots light and old dark) and dark). The maximum order of branching indicated the maximum position of a root within the branched hierarchy of the root system (Eissenstat et al. 2000; Wells & Eissenstat 2003).

The shape of the root nodules was classified according to Corby (1971, 1988). The nodules were classified according to their size as more than 4 mm, 2–4 mm, or less than 2 mm in diameter (Patreze & Cordeiro 2005).

Data analysis

Intraspecific variability in root morphology was evaluated using cluster analysis in the STATISTICA 8.0 program (Statsoft, Tulsa). This analysis was based on the mean values of all measured root traits for individual species. Individual categorical variables were coded in the form of dummy variables. The ordination approach was used to evaluate interspecific as well as intraspecific variability together. Detrended Correspondence Analysis (DCA) was used to evaluate total variability and the results were visualized in the form of an ordination diagram. Redundancy Analysis (RDA) was used to evaluate the proportion of root system variability explained by environmental variables and by species effect. Standardization by species (dependent variables) was used because the analysed data were of various types and units. The statistical significance of the first and all constrained canonical axes was determined by the Monte Carlo permutation test. In all analyses, 999 unrestricted permutations were used. All ordination analyses were performed in the CANOCO for Windows 4.5 program (ter Braak & Šmilauer 2002).

Results

Over three years, 214 plants of 21 legume species were excavated and measured. The mean values of the investigated root traits for all studied species are given in Table 1. The investigated species belonged to five

Table 1. Summary of root morphology and nodule traits.

Species	Abbreviation	Tribus	Number of plants	Number of sites	Life forms	Type of root system	Depth [cm]	Rhizome	Maximum order of branching	Recovery buds	Special diameter [mm]	Woodiness	Colour	Shape of nodules	Size of nodules [mm]
Anthyllis vulneraria	AntVul	*Loteae*	5	1	H	RS	>30	0	3	no	3–4	yes	dark	a	<2
Astragalus glycyphyllos	AstGly	*Galegeae*	7	2	H	RS	>30	0	5	yes	3–4	no	near–white	b, c, e	> 4
Cytisus scoparius	CytSco	*Genisteae*	6	3	N	RS	>30	0	5	yes	7–8	yes	dark	b, c	> 4
Genista tinctoria	GenTin	*Genisteae*	11	1	N	HR	10–20	1	5	yes	5–6	yes	dark	a, b, c, e	> 4
Lathyrus pratensis	LatPra	*Viciaea*	11	6	H	HR	20–30	1	3	no	3–4	yes	light/dark	c	< 2
Lathyrus sylvestris	LatSyl	*Viciaea*	13	2	H	HR	20–30	1	3	yes	11–12	yes	light/dark	c	2–4
Lotus corniculatus	LotCor	*Loteae*	16	5	H	RS	>30	1	3	yes	5–6	yes	dark	a	<2
Lupinus polyphyllus	LupPol	*Galegeae*	11	3	H	RS	>30	0	3	yes	10–11	no	light/dark	d	> 4
Medicago lupulina	MedLup	*Trifolieae*	10	2	H	RS	>30	0	3	no	3–4	yes	light/dark	c, e	> 4
Medicago sativa	MedSat	*Trifolieae*	10	2	H	RS	>30	0	3	yes	5–6	yes	dark	c, e	> 4
Melilotus albus	MelAlb	*Trifolieae*	5	1	H	RS	20–30	0	5	no	8–9	no	light	c, e	> 4
Melilotus officinalis	MelOff	*Trifolieae*	5	1	H	RS	20–30	0	5	no	8–9	no	light	c, e	> 4
Securigera varia	SecVar	*Loteae*	12	3	H	RS, HR	>30	0	3	yes	3–4	yes	dark	c, e	2–4
Trifolium arvense	TriArv	*Trifolieae*	29	3	T/H	RS	0–10	0	3	no	1–2	no	near–white	c, e	<2
Trifolium campestre	TriCam	*Trifolieae*	6	3	T/H	RS	0–10	0	3	no	1–2	no	near–white	c	<2
Trifolium medium	TriMed	*Trifolieae*	6	3	H	RS	20–30	1	4	no	3–4	yes	light/dark	c	<2
Trifolium pratense	TriPra	*Trifolieae*	21	3	H	RS	20–30	0	3	no	3–4	no	near–white	c	<2
Trifolium repens	TriRep	*Trifolieae*	6	3	H/Ch	HRS	10–20	1	3	no	1–2	no	near–white	c	<2
Vicia angustifolia	VicAng	*Viciaea*	7	3	T/H	RS	10–20	0	3	no	1–2	no	near–white	c	<2
Vicia cracca	VicCra	*Viciaea*	12	3	H	HR	20–30	1	3	no	3–4	yes	dark/white	c, e	> 4
Vicia hirsuta	VicHir	*Viciaea*	5	4	T/H	RS	0–10	0	3	no	1–2	no	near–white	c, b, e	2–4

Life forms according to Raunkiaer (1934): H – hemicryptophyte; N – nanophanerophyte; T – terophyte; Ch – chamaephyte; type of root system: RS – root splitters, HR – hypogeogenous rhizomes, HRS –horizontal rooting stems or above the soil surface; special diameter – mean value of taproot diameter under the crown and maximum diameter within the root system; shape of nodules: a – globose, b – branched, c – cylindrical, d – ruff-like, e – fan-like.

tribes (*Galegeae*, *Genisteae*, *Loteae*, *Trifolieae* and *Vicieae*), four life forms (hemicryptophyte, chamaephyte, nanophanerophyte and therophyte), and three types of root system (root splitter – Fig. 1; hypogeogenous rhizomes – Figs 2a,b; horizontal rooting stems on or above the soil surface – Figs 2c,d). The depth of the root systems ranged from 0 to 10 cm in three annual species, from 10 to 20 cm in one annual and five perennial species, from 20 to 30 cm in four perennial species, and was more than 30 cm in seven perennial species. A rhizome was present in seven species and the maximum order of branching was three for 15 species, four for one species, and five for five species. Recovery buds were recorded on the root system of eight species and woodiness was recorded in 11 species. Root diameter ranged from 1 to 12 mm – the minimum diameter was recorded in annuals and the maximum in the perennials *Lathyrus sylvestris* and *Lupinus polyphylus.* The colour of the root systems ranged from light to dark. In six species, young roots were light whereas older roots were dark (see Fig. 2a). Globose (Figs 3a,b), cylindrical (Figs 3c,d), branched (Figs 4a,b), fan-like (Figs 4c,d), and ruff-like (Figs 4e,f) nodules were recorded. One type of nodule shape was recorded in 11 species, two types in seven species, and three or four types in three species. Nodules of up to 2 mm in size were recorded in nine species, from 2 to 4 mm in three species, and more than 4 mm in nine species.

The results of the cluster analysis are shown in Fig. 5. The root system of *L. polyphyllus* differed the most from the other investigated species, being separated from the other species in the first division. The main reason for the separation of *L. polyphyllus* was the large size of its root system and nodules in comparison to other species. In addition, roof-like shaped nodules were only recorded in this species (see Figs 4e,f).

Two large groups of species were distinguished in the second division. The main cause of the second division was the light *versus* dark colour of the root systems. The large group on the left was divided into four smaller groups. *Trifolium repens,* the only species in Group 1, had horizontal rooting stems on the soil surface (Fig. 2c). Group 2 included species with rhizomes, such as *Vicia cracca*, *L. sylvestris*, *Trifolium medium* and *Lathyrus pratensis* (Fig. 2a). All these species had light then dark root systems. Group 3 was divided into two sub-groups: *Trifolium pratense* (Fig. 1c) on one side and the annual species *Vicia hirsuta*, *Trifolium campestre*, *Vicia angustifolia* and *Trifolium arvense* (Fig. 1a) on the other. The overall structure of the root systems was similar, but the annuals differed from *T. pratense* by having a flat fibril root system

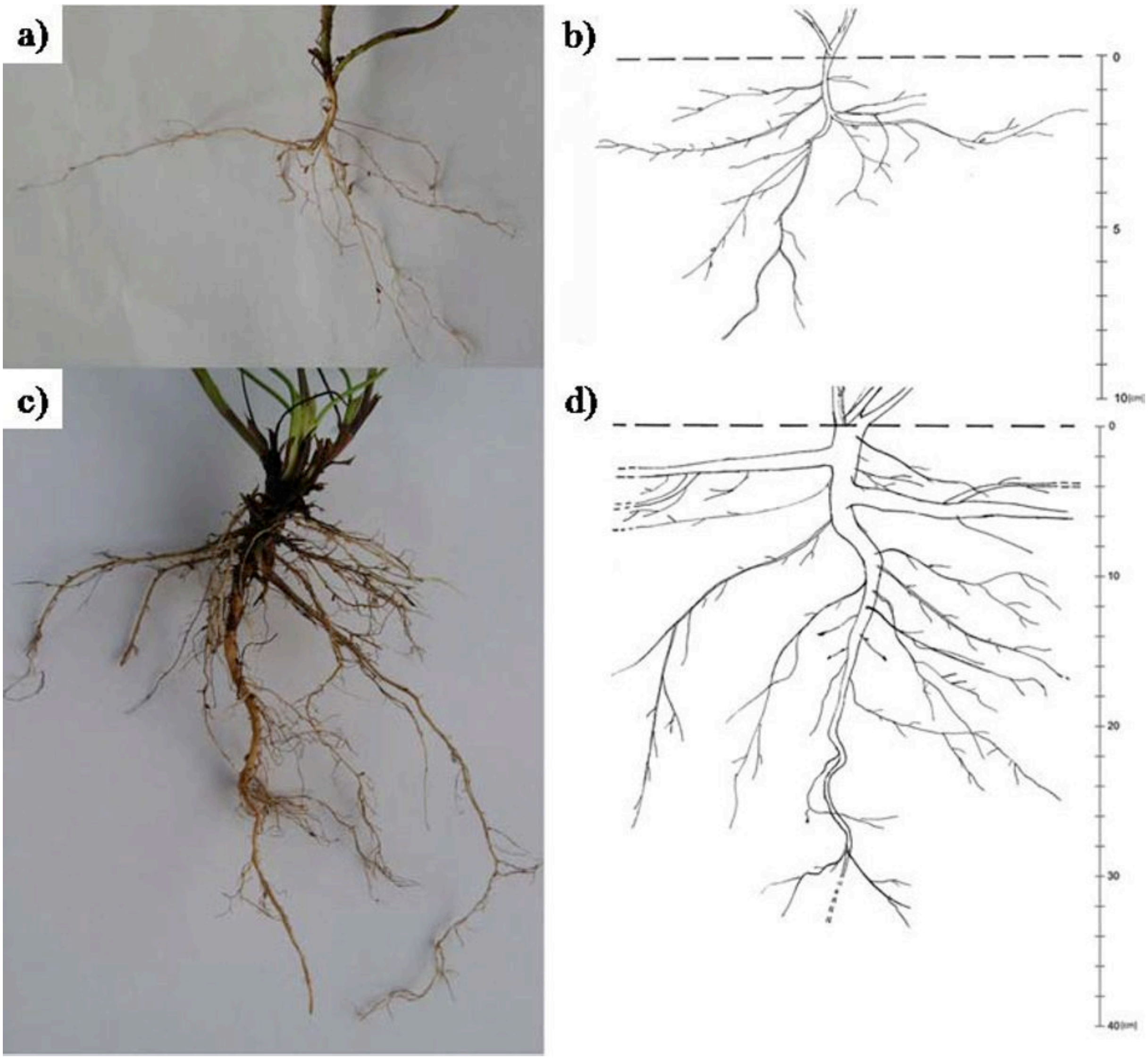

Fig. 1. a – root system of *Trifolium arvense*; b – schematized typical root system of studied annual legumes with "root splitter" root type; c – root system of *Trifolium pratense* and d – schematized typical root system of studied perennial legumes with "root splitter" root type.

spread under the soil surface. The root systems of annuals were more branched than in *T. pratense* and lacked regenerating buds or rhizomes. The clearly visible taproot of *T. pratense* indicated its perennial character. Group 4 was composed of species with a taproot and larger nodules than in the previous groups. Another typical feature of legumes in Group 4 was the presence of more shapes of nodules in each species. The righthand group following division number two included perennial species with a well-developed taproot. Species in Group 5 differed from species in Group 6 (division number 4) by the shape of their nodules (globose, fan-like, cylindrical and branched *versus* globose) and by their larger size. In Group 5, *Securigera varia* and *Medicago sativa* were strictly separated from nanophanerophytes *Genista tinctoria* and *Cytisus scoparius*. Group 6 contained species from the tribe *Loteae* (*Lotus corniculatus* and *Anthyllis vulneraria*). A typical feature of these species was nodules of up to 2 mm in size.

The results of DCA (Fig. 6) corresponded well with the results of the cluster analysis as the same groups of species were distinguished. As in the cluster analysis, *L. polyphyllus* was separated from all other species. Intraspecific variability in this species was high, mainly due to the different sized root systems in the measured plants.

L. corniculatus and *A. vulneraria* (Group 6) had the lowest intraspecific variation in root systems as all investigated plants were close together in the ordination diagram. On the other hand, *S. varia* had the highest interspecific variability in the root system. This was because individuals with root splitter (55% cases) and hypogeogenous rhizome (45% cases) root types were recorded in this species. Further variability in the root systems was due to the presence of more than one shape of nodule on one plant (cylindrical and fan-like) and their variable size (2–4 mm). The transition between perennial species with rhizomes (Group 2) and annual species with a taproot (Group 3) was shown by *T. repens*, which has horizontal rooting stems (Group 1).

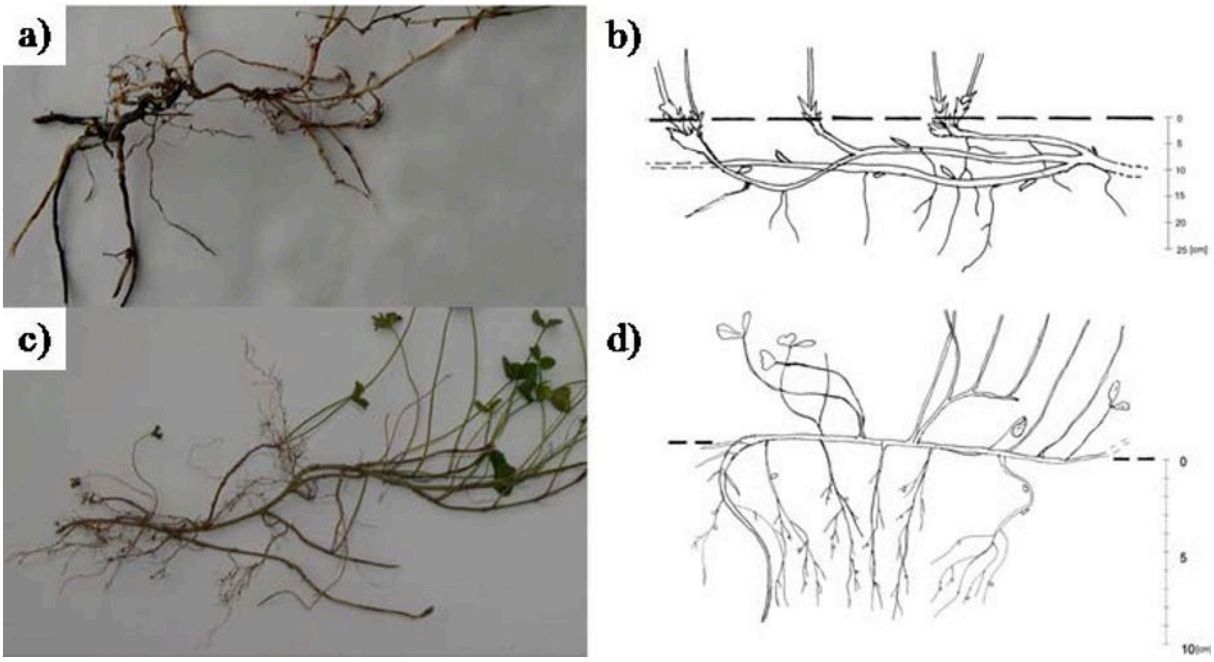

Fig. 2. a – root system of *Lathyrus pratensis* with light/dark colour; b – schematized typical root system of legumes with rhizomes – "hypogeogenous rhizomes"; c – root systems of *Trifolium repens*;and d – schematized horizontal rooting stems on or above the soil surface.

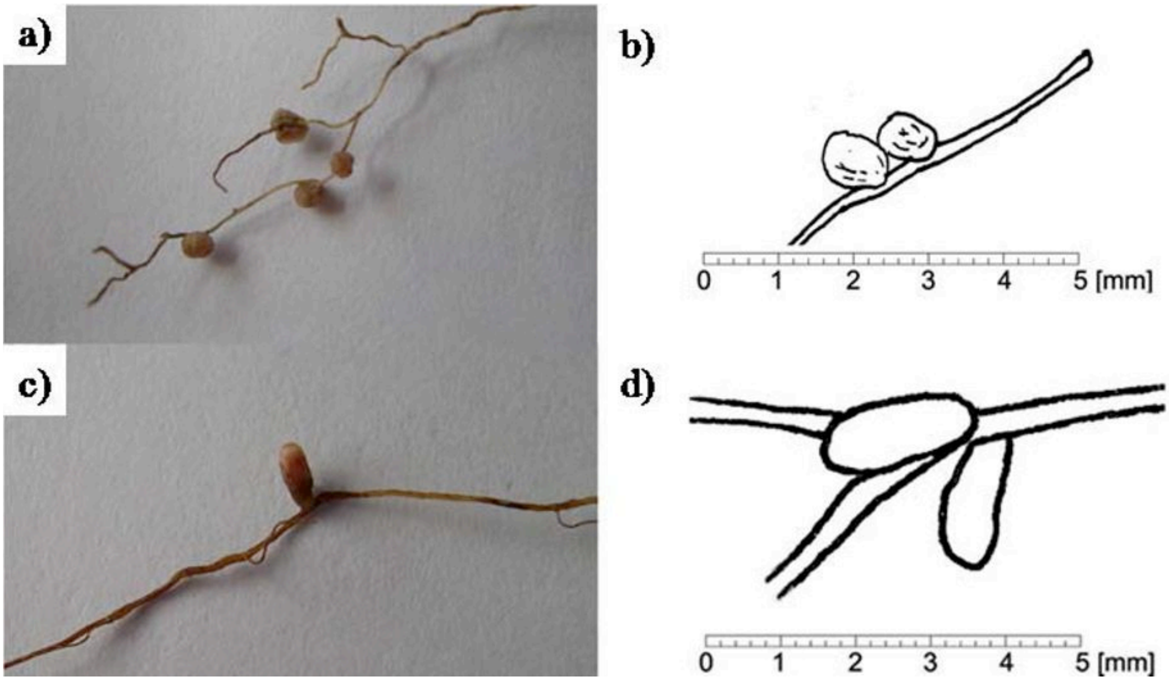

Fig. 3. a – nodule shape of *Lotus corniculatus*; b – schematized globose nodules; c – nodule shape of *Lathyrus pratensis*; d – schematized cylindrical nodules.

Interspecific variability in the root systems of *T. repens* was low.

With the exception of two individuals, root system variability was low in *T. arvense.* The typical shape of nodules for all investigated *Trifolium* species was cylindrical, but the two most distinct individuals of *T. arvense* had fan-shaped nodules. In species with more than one shape of nodule, not all recorded shapes were found on all individuals. This substantially increased the intraspecific variability of the root systems in *Astragalus glycyphyllos*, *C. scoparius*, *G. tinctoria*, *Medicago lupulina*, *M. sativa*, *Melilotus albus*, *Melilotus officinalis*, *S. varia*, *T. arvense*, *V. cracca* and *V. hirsuta.*

The intraspecific variability in annual species (*T. arvense*, *T. campestre*, *V. angustifolia* and *V. hirsuta*) and *T. repens* was due primarily to the depth of the root systems. The special diameter was highly variable in *A. vulneraria*, *C. scoparius*, *L. pratensis*, *M. albus*, *M. of-*

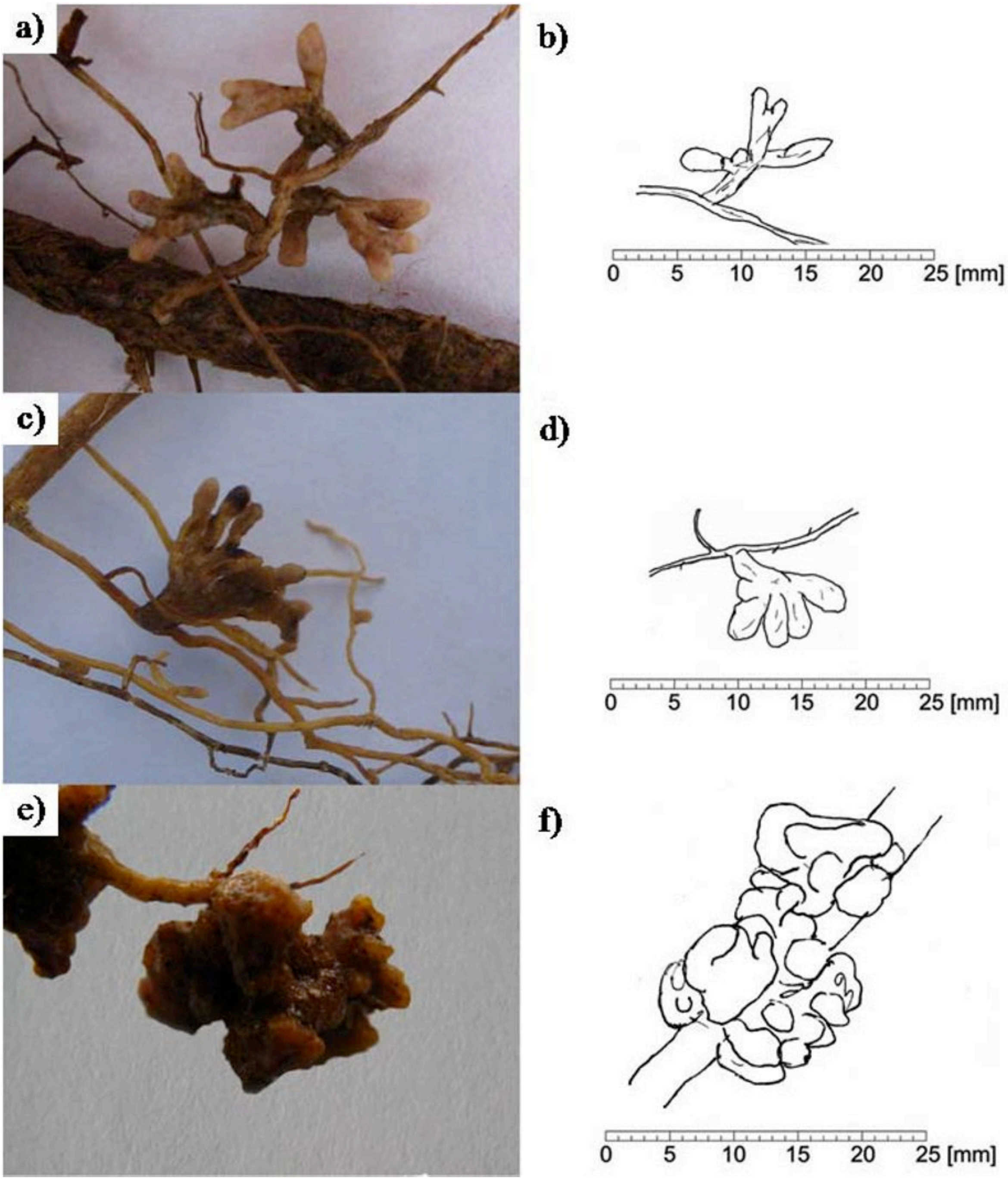

Fig. 4. a – nodule shape of *Lathyrus sylvestris*; b – schematized branched nodule; c – nodule shape of *Vicia cracca*; d – schematized fan-like nodule; e – nodule shape of *Lupinus polyphyllus*; f – schematized ruff-like nodule.

ficinalis and *T. medium*. The special diameter together with the maximum order of branching was variable in *L. pratensis*, *L. polyphyllus*, *M. lupulina*, *M. sativa*, *S. varia* and *T. pratense*.

The results of the redundancy analyses are shown in Table 2. The environmental variables (dry or humid site, field, forest, ruderal or meadow, shaded or sun exposed, stony or without stones in the upper 30 cm soil layer, with low or high cover of herbaceous vegetation) together explained 48.9% (analysis a1) of the variability in root systems.

Forward selection was employed to select only the environmental variables that significantly improved the model. The total number of 11 environmental variables was reduced to three, i.e. field, shade and drought, which together explained 47.1% of the variability (analysis a2). The variability explained by field, shade and drought was only 17.4% after the effect of species was removed from the analysis (analysis a3).

The highest proportion of root system variability was explained by the effect of species – species alone

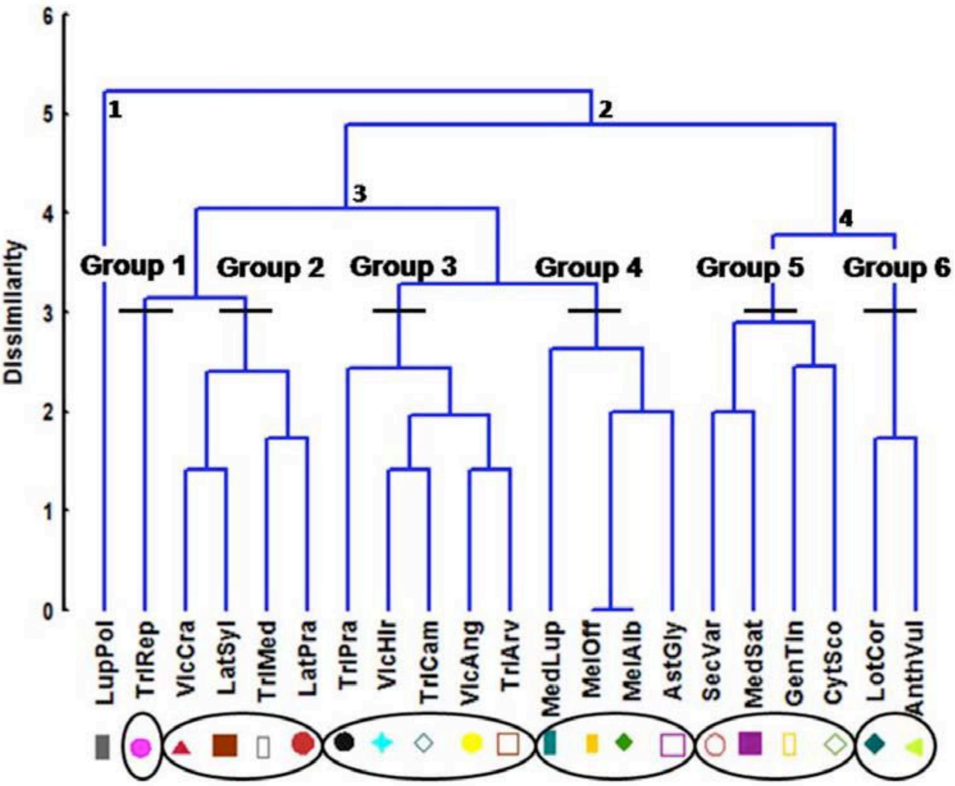

Fig. 5. Dendrogram based on cluster analysis representing interspecies variability between 21 investigated legume species. The numbers refer to divisions described in the text. Species abbreviations are given in Table 1. The labels for individual species are consistent with Fig. 6.

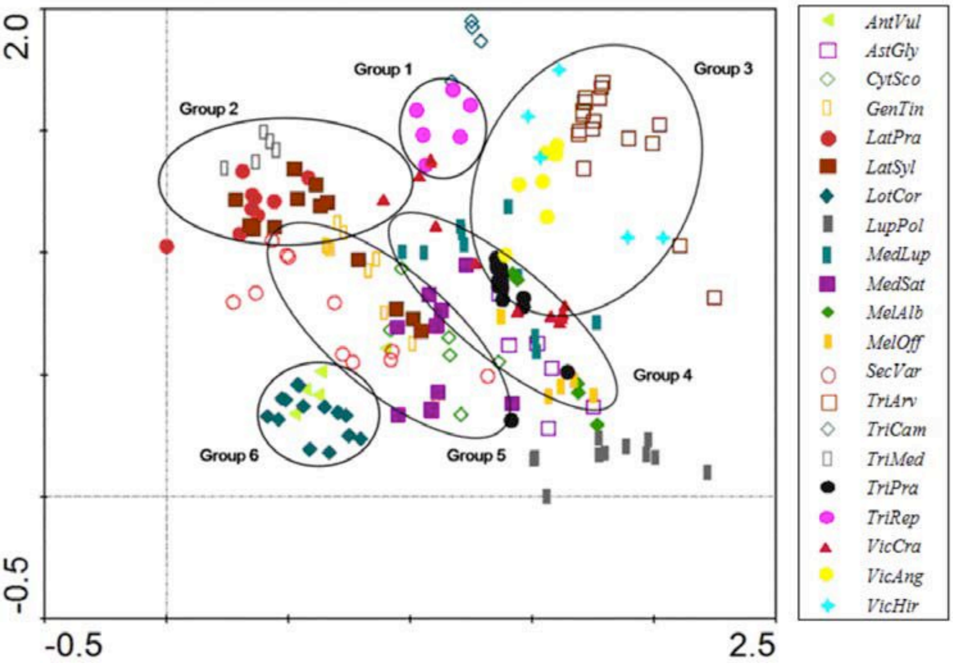

Fig. 6. Ordination diagram of the Detrended Correspondence Analysis (DCA) reflecting the intraspecific and interspecific variability in the root systems of 21 investigated legume species. Species abbreviations are given in Table 1. The labels for individual species are consistent with Fig. 5.

explained 44.9% of the root system variability (analysis a5), and this decreased only marginally when variability caused by drought, field and shade was removed from the model (analysis a6).

Discussion

Although the root systems of legumes have frequently been studied in laboratory and field conditions, there is

Table 2. Results of redundancy analyses of root systems data.

	Expl. Var.	Covariables	% ax 1 (all)	F 1 (all)	P 1 (all)
a1	C, D, F, Fo, Mo, R, S, St, H, Species		21.5 (48.9)	49.81 (16.12)	0.001 (0.001)
a2	D, F, S		20.0 (47.1)	47.55 (19.55)	0.001 (0.001)
a3	C, D, F, Fo, Mo, R, S, St, H	Species	13.0 (17.4)	27.23 (3.74)	0.001 (0.001)
a4	D, F, S	Species	7.9 (22.0)	16.28 (6.49)	0.001 (0.001)
a5	Species		18.4 (44.9)	43.41 (19.82)	0.001 (0.001)
a6	Species	D, F, S	16.7 (43.8)	38.08 (19.29)	0.001 (0.001)

An. – analysis code; Expl. var. – explanatory (environmental in Canoco terminology) variables; % ax 1 (all) – species variability explained by canonical axis 1 or by all axes in brackets (measure of explanatory power of the environmental variables); F 1 (all) – F statistics for the test of axis 1 or all axes in brackets; P 1 (all) – corresponding probability value obtained by the Monte Carlo permutation test for the test of axis 1 or all axes in brackets. Environmental variables and covariables: C– cover of vegetation above 40%; D – dry site; F – site with field conditions; Fo – forest; H – humid site; M – meadow; R – ruderal site; S – shaded site; St – stony site in upper 30 cm of the soil profile. Species were coded in the form of 21 dummy variables.

a lack of studies covering a wide range of species in natural conditions. Therefore a unique feature of this study is the wide range of investigated species collected from different environmental conditions, thus demonstrating the natural variability of leguminous root systems.

The effect of species and environment on root system variability was not fully separated in this study performed at the landscape level because not all investigated species were present at all localities. Therefore there was much overlap between the root system variability explained by the effect of species and by environmental conditions. This was clear from the large reduction in variability explained by the environment when the variability caused by effect of species was removed from the redundancy analysis by covariables. Despite being unable to strictly separate the variability caused by species and by the effects of the environment, large differences in root systems were recorded between particular species. This was supported by the fact that species recorded together at one locality frequently had different root systems. The variability in root systems within each species was substantially lower than that of the whole data set, as indicated by clusters of points for individual species in the ordination diagram (Fig. 6). The restricted variability of the root system for individual species compared to all species together is consistent with the results of Galloway & Fenster (2000).

Of all the recorded environmental variables, root system traits were the most affected by moisture and light availability and by field conditions. This is consistent with studies dealing with the effect of environmental conditions on root systems (Koukoura et al. 2009; Salako et al. 2002; Šmilauerová & Šmilauer 2002; Wahl et al. 2001). Further, it must be noted that the species present at dry sites frequently differed from species recorded at wet sites, and therefore it is impossible to discuss the effect of environmental conditions on the root system variability of individual species in more detail.

Although the variability caused by site and species effect cannot be strictly separated, the intraspecific variability of legume root systems was substantially lower than the variability within all species. However, the root systems of many legume species may differ even when growing in the same environmental conditions, and this difference is genetic. It must be noted that, to compare the intra- and interspecific variability of all studied species exactly, all investigated species must be present at all studied localities, and this can only be achieved via transplantation.

The intraspecific variability in root systems was due to Raunkier's life forms rather than tribes, since in several cases species with different life forms were recorded in the same tribe. For example, annual and perennial species with highly different root systems were both recorded in the tribes *Trifolieae* and *Viciaea.* On the other hand, species in the tribes *Loteae*, *Galegeae* and *Genisteae* were all perennial and therefore had relatively low root system variability. The differences between root systems of annuals and perennial species were also recorded by Fitter et al. (1988), Gross et al. (1992) and Roumet et al. (2008).

Annual root systems were intermediate between perennial rhizomatic and perennial species with a taproot. This is clearly visible in Fig. 5, in which annuals (*T. arvense*, *T. campestre*, *V. angustifolia* and *V. hirsuta*) are located in the middle of the dendrogram between rhizomatic and taproot perennials. The relation between differences among species and life form is consistent with the results of Gross et al. (1992).

The intraspecific variability in root systems probably enables better survival of individual plants in different environmental conditions. The individual traits of root systems differed in terms of their plasticity under various environmental conditions, e.g. branching was more plastic than special root diameter. The same result was recorded by Forde & Lorenzo (2001), indicating that root diameter may be highly species-specific and therefore highly heritable, while branching may depend more on soil conditions at the level of the individual plant. We concluded that some root traits are determined more by environmental conditions and others by heritability. This conclusion is in accordance with the results of Johnson et al. (1996). In *A. vulneraria*, *A. glycyphyllos*, *L. corniculatus*, *M. sativa* and *S. varia*, differences in branching contributed the most to the large differences among individual plants within the species. The colour of the root systems depended on the life form: most of the root systems were pale brown and this colour was recorded in all annuals and in the young roots of perennials. According to Corby (1971), pale brown is the most frequent root colour. Rejili et al. (2007) suggested that the colour of the roots is dependent on the nodules and the leghaemoglobin content. The latter was not investigated in our study, nor was the dependence of colour on nodulation recorded.

The most frequently recorded shape of nodules was cylindrical. Small globose nodules were considered juvenile and thus were ignored by Corby (1971), but not in our study. We recorded all shapes of nodules, because small nodules can be wrongly considered as juvenile, especially in species with small nodules. For example, small globose nodules were typical in *L. corniculatus* and *A. vulneraria*, and small cylindrical nodules were found in all *Trifolium* species. According to Corby (1988), one shape of nodule may be typical for a particular tribe or there may be tribes in which more nodule shapes are possible, but only several of these are dominant. Our results indicate that more than one shape of nodule is present in some tribes (*Galegeae*, *Genisteae* and *Viciaea*), but that often only one shape is dominant. In our study, most nodules were located on the lateral roots, which is consistent with the results of Capoen et al. (2010). The shapes of the nodules were highly species-specific, as reported previously by Rejili et al. (2007). The exceptions were two plants of *T. arvense*, which had fan-like nodules of a different size than all other *T. arvense* specimens. A possible explanation is that different species of bacteria had created these nodules, but this requires further investigation. It is possible that these two plants were infected by bacteria from *S. varia* growing in their vicinity, because the nodules of *S. varia* were fan-like. The differences in root systems may be related to plant taxonomy, but the taxonomy of *S. varia* is not entirely clear. According to Allen & Allen (1981), *S. varia* belongs to the tribe *Loteae*. In our study, nodules other than those that are typical for this tribe were recorded in *S. varia*, indicating that *S. varia* could be included in the tribe *Coronilleae* rather than *Loteae*. The transitional character of *S. varia* on the border between these two tribes was also discussed by Allan & Porter (2000).

The nodules of *L. polyphyllus* were the most different from all other species, which is consistent with the classification suggested by Corby (1988).

Eight of studied perennial root splitters (*A. vulneraria*, *A. glycyphyllos*, *C. scoparius*, *L. corniculatus*, *L. polyphyllus*, *M. lupulina*, *M. sativa* and *S. varia*) reached a rooting depth of more than 30 cm. This is consistent with the description of these species in plant trait databases, as all these species are described as deep rooting (Fitter & Peat 1994). On the other hand, all annuals (*T. arvense*, *T. campestre*, *V. angustifolia* and *V. hirsuta*), rhizomatous perennials (*G. tinctoria*, *L. pratensis*, *L. sylvestris*, *S. varia* and *V. cracca*) and some root splitter perennials (*L. polyphyllus*, *M. albus*, *M. officinalis*, *T. medium* and *T. pratense*) were shallow rooting, reaching depths of up to 30 cm. During plant excavation, we recorded most of the fine roots of all investigated legumes in the shallow-rooted species. This is probably an adaptation to drought, as described by Merrill et al. (2002).

We can conclude that the root systems of common European legumes are highly variable. The variability of root systems was attributed to Raunkier's life forms rather than the membership of species in tribes.

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II. Effect of nitrogen, phosphorus and potassium availability on emergence, nodulation and growth of acidicole *Trifolium arvense* L. in alkaline soil

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Effect of nitrogen, phosphorus and potassium availability on emergence, nodulation and growth of acidicole *Trifolium arvense* L. in alkaline soil

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ABSTRACT

The acidicole behavior of many species is given by their inability to acquire enough P in alkaline soil, but reasons for acidicole (syn. calcifuge) behavior of *Trifolium arvense* have never been studied experimentally. We asked how emergence of seedlings, the survival of plants, the growth of aboveground organs, the nodulation and the growth of roots of this species is affected by different N, P and K supply in alkaline soil. In the years 2010 and 2011, we performed a pot experiment (ten N, P and K fertilizer treatments) with seeding of *T. arvense* into alkaline soil. The acidicole behavior of *T. arvense* proved to be connected with the inability of seedlings to acquire enough P during their emergence. In all treatments, *T. arvense* was not able to flower in the seeding year and in the second year it flowered only in P treatments. This indicates the biennial character of the species in the case of late seeding and points to a strong P limitation of flowering and seeds production in plants grown on alkaline soil. Without P addition, *T. arvense* was not able to develop beyond the seedling stage. Nodulation was positively affected by P application and negatively by N application. Although *T. arvense* is a typical species for P poor soils, it does not suffer from P toxicity under high P supply. We concluded that the acidicole behavior of *T. arvense* is based on its P limitation when growing on alkaline soil.

Introduction

Trifolium arvense L. (haresfoot clover) is an erect or ascending annual or biennial legume native of Europe, 10–30 cm tall, usually with spreading branches (Rothmaler et al., 2000; Slavík, 1995), and roots reaching to a depth of 10 cm (Fitter and Peat, 1994; Klimešová and de Bello, 2009). This legume preferably occurs on sun-exposed sites with low soil moisture, low nutrient availability and acid soil reaction (Ellenberg et al., 1992) and is a typical species for pioneer vegetation on sandy and shallow soils in Central Europe (Chmelíková and Hejcman, 2012; Chytrý, 2007). The life strategy (sensu Grime, 2001) is intermediate between ruderal (R) and stress-tolerant (S).

A question which has never been investigated is why *T. arvense* prefers acid and low productive soils. Tyler (2003) suggested that the acidicole (syn. calcifuge) behavior of many species could be explained by their inability to acquire enough P, which is present in different forms in alkaline rather than in acid soils. An alternative explanation of the acidicole behavior of many species could be their inability to acquire enough Fe, Mn or Zn from an alkaline soil, as all these elements are well plant-available on acid soils, but scarcely so on alkaline soils (Lambers et al., 2008; Shane et al., 2008). This explanation of acidicole/acidifuge (syn. calcifuge/calcicole) behavior of species is based on results of transplantation experiments where many acidicole species transplanted on alkaline soil performed well when P or Fe fertilizers were supplied (Tyler, 1994, 1996).

Legumes are well known for their high P and K requirements, but low mineral N requirements, as they are able to fix sufficient amounts of N via symbiotic bacteria (Howieson et al., 2011; Li et al., 2011; Rochon et al., 2004; Tang et al., 1999). Positive responses of *T. arvense* on addition of P were reported by Dodd and Orr (1995) in soils with low pH, but no information is available of whether the same response can be recorded in an alkaline soil.

Many stress tolerant species are well adapted to P-poor conditions as they probably have evolved under low P availability in the soil, as low P availability was a typical feature of many natural, old soils (Lambers et al., 2008). High P availability is frequently toxic for species tolerant to edaphic stress, as no mechanisms of protection against high P supply were necessary during their evolution (Pang et al., 2010). It has not yet been studied, whether high P availability is also toxic for stress tolerant legumes such as *T. arvense*.

The soil reaction affects the survival and persistence of *Rhizobium* s.l. bacteria and their nodulation on roots, and therefore also the ability of legumes to fix aerial N_2 (Graham and Vance, 2000;

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Table 1
Fertilizer treatments and amount of nutrients applied in one dressing. Numbers given in brackets correspond to the total seasonal amount of applied nutrients (two dressings per season).

Treatment	N [kg ha^{-1}]	P [kg ha^{-1}]	K [kg ha^{-1}]
N1	150 (300)	–	–
N2	300 (600)	–	–
P1	–	40 (80)	–
P2	–	80 (160)	–
K	–	–	100 (200)
N1P1	150 (300)	40 (80)	–
N1P1K	150 (300)	40 (80)	100 (200)
N2P1K	300 (600)	40 (80)	100 (200)
N2P2K	300 (600)	80 (160)	100 (200)
Control	–	–	–

Zahran, 1999). It can be supposed that preference of *T. arvense* for acid soils might be connected with low root nodulation rates in alkaline soils, leading to low ability to acquire enough N for growth.

Soil reaction and nutrient availability can highly affect seed germination and seedling emergence. Seedlings frequently require a higher P supply than adult plants, as was recently demonstrated for *Rumex crispus* and *R. obtusifolius* by Křišťálová et al. (2011). Higher sensitivity of seedlings than of adult plants to P shortage can be decisive for their survival, but this has never been investigated in the case of *T. arvense*. Further, no flowering of several acidicole species transplanted on alkaline soil was recorded by Tyler (1996), but *T. arvense* was not included in this group of plants.

Using a pot experiment with adding fertilizer to an alkaline soil, the main aim of this study was to investigate the performance of the principally acidicole *T. arvense* under such conditions. Within this context, we asked how the seedlings emergence, the survival of plants, the nodulation, the growth of roots, the growth of aboveground organs and the seed production are affected by different N, P and K availability in an alkaline soil.

Materials and methods

Design of the experiment

In April 2010, a pot experiment was established in an open-air vegetation hall in the Crop Research Institute in Prague (Czech Republic, 50°5′N; 14°18′E) with natural rain, temperature and light conditions. The experiment was completely terminated in September 2011. Pots were fertilized using a combination of two levels of N addition (N1 and N2), two levels of P addition (P1 and P2) and one level of K addition (K). Fertilizers were applied in 2010, but not in 2011. This resulted in ten different treatments: N1, N2, P1, P2, K, N1P1, N1P1K, N2P1K, N2P2K and a control without any fertilizer input (see Table 1 for details). Each treatment was replicated five times (50 pots in total). The pots were fertilized twice per vegetation season, on 20 April and 27 July, using the following fertilizers dissolved in water: ammonium nitrate with lime ($NH_4NO_3 + CaCO_3$, containing 27.5% N, 10% Ca), super phosphate ($Ca(H_2PO_4)_2 + CaSO_4$, 8.5% P, 20% Ca, 10% S) and potassium chloride (KCl, 50% K, 47% Cl).

The pot volume was 30 L and the pot surface area was 1963 cm^2 (pot diameter 50 cm). Clay soil with the following chemical properties was used: $N_{total} = 614\ mg\ kg^{-1}$, $K_{Mehlich\ III} = 62\ mg\ kg^{-1}$ (low K availability), $P_{Mehlich\ III} = 16\ mg\ kg^{-1}$ (low P availability), $Mg_{Mehlich\ III} = 422\ mg\ kg^{-1}$, $Ca_{Mehlich\ III} = 6777\ mg\ kg^{-1}$ and pH (H_2O) = 9.14. The mean temperature from May to October 2010 was 14.4 °C.

Data collection

Seedling emergence

Seeds of *T. arvense* were collected in August 2009 in Western Bohemia. The collection sites were low productive grasslands and roads on sandy soils. Seeds were collected from groups of plants at four localities and mixed to obtain one representative seed sample. Twenty plants were selected randomly at each site, taking care not to favor tall or small plants. The seeds were stored at room temperature, in paper bags in the dark, before start of the experiment.

Fifty seeds of *T. arvense* were sown into each pot with pre-fertilized soil on 5th May 2010 and the cumulative number of seedlings (field emergence) was recorded up to 22nd May as no further increase in number of seedlings was recorded after this date. The depth of sowing was 1 cm and pots were watered if necessary to maintain optimal growth conditions. Heavy rains occurred from 24th May to 2nd June, so that few only plants survived. Therefore we are using this experiment only to characterize the germination success under the different fertilizer treatments. We started thereafter the experiment again, seeding the same amount of seeds on 7th July 2010. The cumulative number of seedlings (field emergence) was then recorded up to 4th August. To be sure that no viable seeds were present in the soil from the first seeding on 5th May, we removed the upper 2 cm of the soil before the second seeding on 7th July.

Number of living plants in October

On 10th October 2010, the number of plants in each pot was counted and expressed as percentage of the total number of used seeds (50) per each pot to make obtained values directly comparable with field emergence in May and in August.

Aboveground organs

On 10th October 2010, five individual *T. arvense* plants per pot were marked and the following traits of them were recorded: (1) height of the plants; (2) number of leaves per plant; (3) diameter of the leaf rosette; (4) length of the terminal leaflet blade; and (5) length of the leaf petiole.

Underground organs

On 10th October 2010, five plants per pot were carefully removed from each pot and their underground organs were floated in water. We selected representative plants in each treatment, taking care not to select the smallest or, on the other hand, the tallest plants. If possible, we left several plants in pots to observe their flowering in the following year, but this was not possible for all treatments as the number of plants was very small in some treatments. Outlines of the plants were digitalized using the scanner. Three traits of the root systems were recorded: (1) length of the taproot; (2) diameter of the root neck and (3) maximal order of branching. In addition to root characteristics, five traits of nodules were recorded: (1) the number of nodules per plant; (2) the length of nodules; (3) the width of nodules; (4) the shape of nodules and (5) their color. The maximal branching order presented the highest developmental branching order in hierarchy of the root system (Eissenstat et al., 2000; Fitter, 1987; Wells and Eissenstat, 2003). We therefore refer to roots with no dependent laterals as 1st-order, roots with a single set of dependent laterals as 2nd-order and so on. The shape of root nodules was classified according to Corby (1971, 1988) as cylindrical, branched, and fan-like shaped, respectively (see Fig. 1). We recorded the proportion of plants with presence of particular nodule shapes from the total number of plants per treatment. For example, 100% of cylindrical nodules indicate that cylindrical nodules were recorded on all plants in the particular treatment. The color of nodules was described as white (inactive), pink (active) or green (moribund or senescent) and expressed in

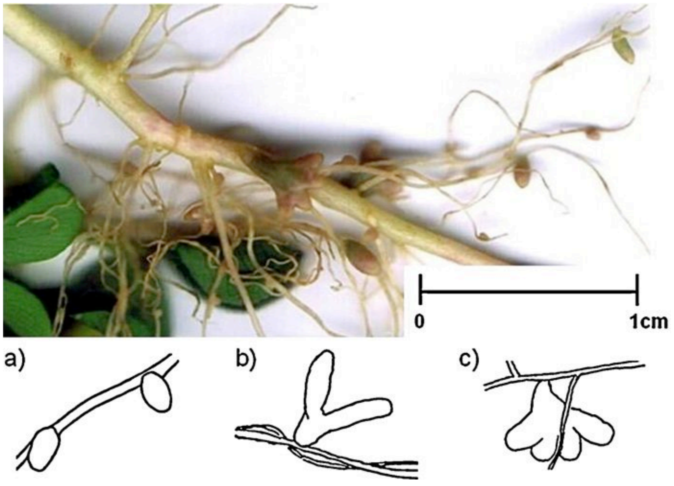

Fig. 1. Classification of nodules into (a) cylindrical, (b) branched and (c) fan-like shaped. A fan-like nodule is clearly visible in the center and a cylindrical nodule in the upper right hand corner of the photograph of part of a *Trifolium arvense* root system.

percentage relative to that of all plants of a treatment. As nodules with three colors were also frequently recorded, there was a high overlap of colors.

Data analysis

One-way ANOVA was used to evaluate the effect of treatment on all collected data. After obtaining significant results, multiple comparisons using Tukey's HSD test were applied to identify significant differences between treatments. All analyses were performed using STATISTICA 7.0 software (Statsoft, Tulsa).

Unconstrained PCA analysis was used to visualize similarity between individual pots, treatments and relationships between measured plant traits separately for aboveground and underground parts of plants. All ordination analyses were performed using the CANOCO for Windows 4.5 program and ordination diagrams were constructed by CANODRAW program (Ter Braak and Šmilauer, 2002).

Results

Seedling emergence

The effect of fertilizer treatment on field emergence recorded on 22nd May and 8th August was significant. On 22nd May, the highest field emergence, 32%, was recorded in P1 followed by P2 treatment and the lowest emergence, 9%, was recorded in the control. Field emergence was intermediate in all other treatments (Fig. 2a). From the second seeding approach, on 8th August the highest field emergence, 42%, was recorded in P2 followed by P1 treatment and the lowest emergence, 8%, was recorded in the control (Fig. 2b).

Number of living plants three months after seeding

On 10th October, the percentage of living plants related to the total number of seeds used during the second seeding, ranged from 10% in N2 up to 40% in P1 treatment and the effect of treatment was significant (Fig. 2c). Taking the number of seedlings on 4th August as the baseline (100%), the number of plants on 10th October increased about 22%, 4% and 39% in N2, P1 and control treatments, respectively. On the other hand, the number of plants decreased about 3%, 8%, 2%, 2%, 33%, 50% and 28% in N1, P2, K, N1P1, N1P1K, N2P1K and N2P2K treatments, respectively, in two months after seedling establishment.

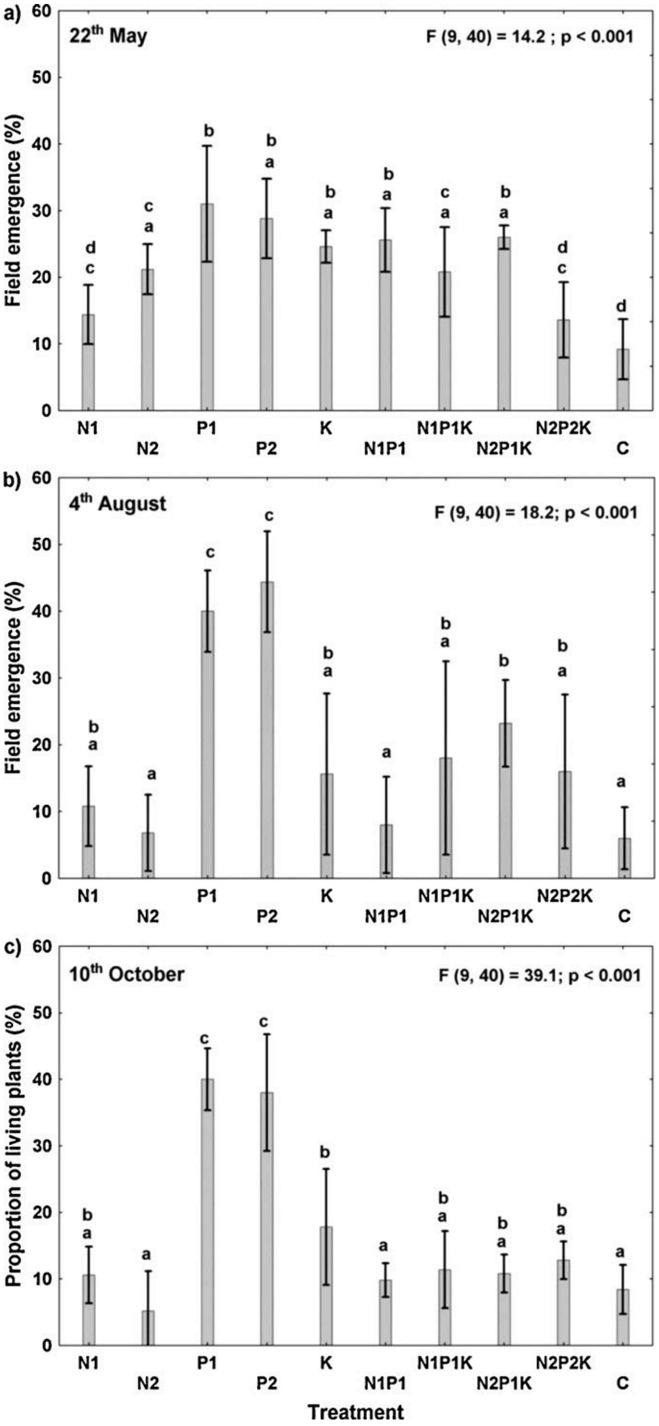

Fig. 2. Effect of different fertilizer treatments on field emergence of *Trifolium arvense* recorded in (a) 22nd May and in (b) 8th August and (c) the proportion of living plants after three months experimental cultivation, related to the total number of initial seeds in each treatment (second seeding approach). Error bars represent standard errors of the means (SE). Treatment abbreviations are given in Table 1. *F* and *P* values in the upper right hand corner of each figure represent results of one-way ANOVA. Using Tukey's post hoc test, treatments with the same letter were not significantly different at the 0.05 probability value.

Aboveground organs

Plant height, number of leaves per the plant, diameter of the rosette, length of the terminal leaflet blade and length of the petiole were highest in P1 and P2 treatments and lowest in all treatments with high N application (N2), K treatment and in the control. In

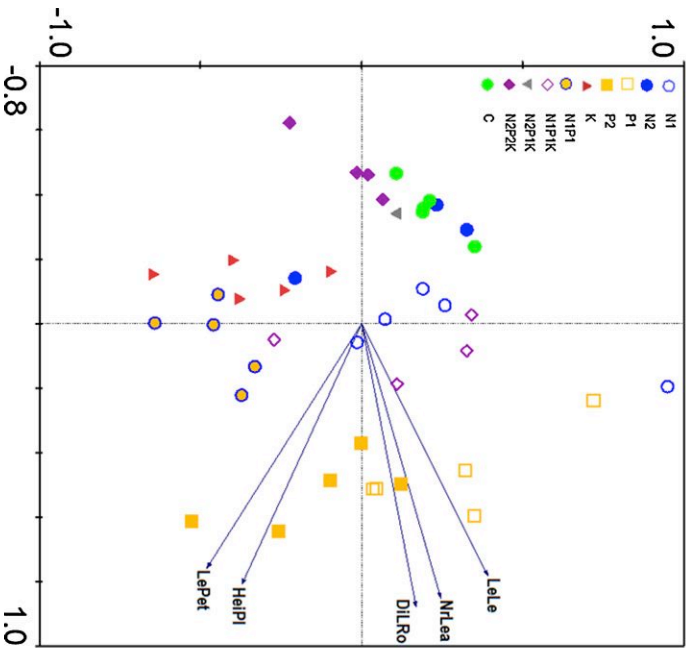

Fig. 3. Ordination diagram showing the results of PCA of aboveground plant traits of *Trifolium arvense* raised under different fertilizer treatments (N1, N2, P1, P2, K, N1P1, N1P1K, N2P1K, N2P2, and Control, see Table 1 for details; 5 separate marks per code: 5 different pots). Plant traits abbreviations: DiLRo – diameter of the leaf rosette, HeiPl – height of the plant, LeLe – length of the terminal leaflet blade, LePet – length of the leaf petiole, NrLea –number of leaves per plant.

all treatments without P application, a violet color indicating P deficiency was recorded. Photographs of plants in all studied treatments are given in electronic appendices 1, 2 and 3. Differences between treatments with the highest and the lowest values for all studied aboveground traits were statistically significant (see Table 2 for details). Differences among treatments and variability among individual pots within one treatment are well visible from the PCA ordination diagram (Fig. 3). The first axis of the PCA explained 66.8% and all axes 95.1% of the data variability. Low variability among individual pots was recorded in control, K, N1P1, N2P2K and N2P1K treatments as marks for individual pots are placed close together. High variability among pots was recorded in N1 and P1 treatments, as one pot in these treatments was highly distant from the remaining four pots. Three groups of treatments were recognized according to their position in relation to the first axis in the ordination diagram. The first group, on the left side of the diagram, is composed of the control and all treatments with N2 application, the second group, in the center of the diagram, is composed of K treatment and all treatments with N1 application, and the third group, on the right side of the diagram, comprises the P1 and P2 treatments. Further, all recorded traits were positively correlated as angles between their vectors are acute in the diagram and they are directed into the same right side of the diagram. Most positively correlated with each other was the height of the plant with the length of the petiole and the number of leaves per plant with the diameter of the rosette.

No stem elongation, flowering and therefore no seed production was recorded in any treatment during the first year of the experiment. Flowering and seed production was recorded, however, in several plants which were left in pots after October 2010 and survived the following winter. Flowering and seed production was recorded only in treatments with P application in 2011.

Table 2
Summary of aboveground and underground traits of *Trifolium arvense* plants. Treatment abbreviations are given in Table 1 One-way ANOVA was applied to evaluate significance of the treatment effect. Using Tukey's post hoc test, treatments with the same letter were not significantly different at the 0.05 probability value.

Treatment	N1	N2	P1	P2	K	N1P1	N1P1K	N2P1K	N2P2K	Control	One-way ANOVA	
											F	*P*
Height of the plant [cm]	4.6^{ad}	2.6^{cd}	6.2^{ab}	8^{b}	5.8^{ab}	5.8^{ab}	6.2^{ab}	1.96^{cd}	1.76^{cd}	1.3^{c}	12.6	<0.001
Number of leaves per plant	14.8^{bcd}	5.4^{ab}	19^{cd}	22.6^{d}	6.2^{ab}	9.8^{abc}	12.4^{abcd}	4.1^{a}	3.8^{a}	5.4^{ab}	9.14	<0.001
Diameter of the leaf rosette	11^{ab}	9.2^{ab}	28^{c}	28.4^{c}	9.8^{ab}	10.68^{ab}	15^{b}	5.3^{a}	7^{ab}	10.6^{ab}	20.18	<0.001
Length of the terminal leaflet blade [mm]	4.82^{c}	3.1^{a}	6.38^{d}	4.74^{c}	2.4^{ab}	3.22^{a}	4.68^{c}	2.66^{ab}	1.3^{b}	1.96^{ab}	28.95	<0.001
Length of the leaf petiole [mm]	8^{a}	5.9^{a}	20.2^{b}	25.2^{b}	10.8^{a}	22.4^{b}	7.7^{a}	6.2^{a}	3.1^{a}	3.6^{a}	22.89	<0.001
Length of the taproot [cm]	6.86^{a}	5.9^{a}	12.32^{a}	8.7^{a}	5.5^{a}	11.1^{a}	8.24^{a}	9.1^{a}	5.6^{a}	5.02^{a}	1.78	0.104
Diameter of the root neck [mm]	1.2^{acd}	0.96^{abc}	1.9^{d}	1.2^{acd}	0.46^{b}	0.98^{abc}	1^{abc}	1.3^{cd}	0.5^{ab}	0.55^{ab}	8.09	<0.001
Maximal order of the branching	3^{ab}	2.4^{a}	2.2^{a}	2.6^{ab}	2^{a}	3.8^{b}	3.8^{b}	2.8^{ab}	2.4^{a}	2^{a}	5.92	<0.001
Number of nodules per plant	3.8^{a}	1^{a}	12.8^{b}	13.4^{b}	5.8^{ac}	8.8^{bc}	11.8^{b}	3.4^{a}	2.4^{a}	5.8^{a}	19.9	<0.001
Length of nodules [mm]	1^{a}	0.6^{a}	3.2^{c}	3^{bc}	0.48^{a}	1.2 ^{a}	1.7^{ab}	1.3^{a}	0.5^{a}	0.9 ^{a}	10.91	<0.001
Width of nodules [mm]	0.56^{ab}	0.5^{ab}	1.7^{c}	1.1^{bc}	0.38^{ab}	0.76^{ab}	0.8^{ab}	0.66^{ab}	0.3^{a}	0.6^{ab}	6.64	<0.001
Cylindrical nodules [%]	80^{a}	20^{a}	100^{a}	100^{a}	80^{a}	100^{a}	80^{a}	60^{a}	60^{a}	40^{a}	2.14	0.048
Branched nodules [%]	0^{a}	0^{a}	100^{c}	80^{bc}	0 ^{a}	60^{abc}	80^{bc}	0^{a}	0^{a}	0^{a}	9.41	<0.001
Fan-like nodules [%]	0^{a}	0^{a}	60^{b}	80^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	8.93	<0.001
White nodules [%]	100^{a}	40^{a}	100^{a}	100^{a}	80^{a}	100^{a}	100^{a}	60^{a}	40^{a}	60^{a}	2.37	0.030
Pink nodules [%]	100^{a}	60^{ab}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	40^{ab}	0^{b}	60^{ab}	6.81	<0.001
Green nodules [%]	40^{a}	60^{a}	100^{a}	100^{a}	100^{a}	80^{a}	100^{a}	80^{a}	20^{a}	40^{a}	3.02	<0.001

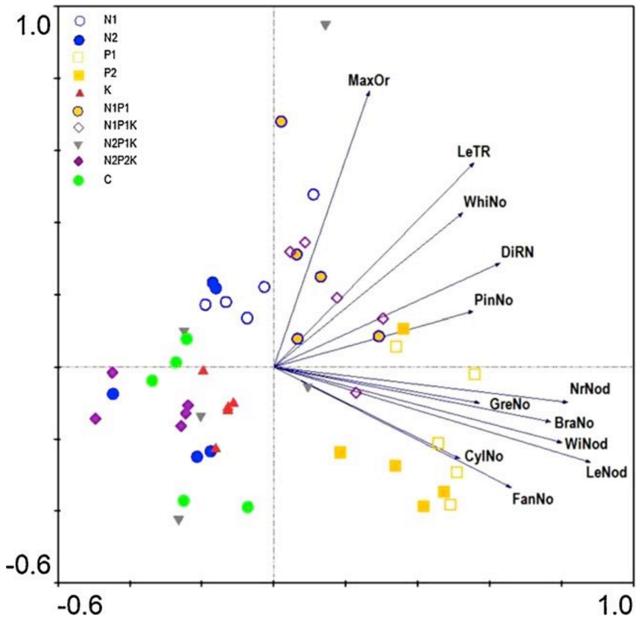

Fig. 4. Ordination diagram showing the results of PCA of underground plant traits of *Trifolium arvense* in ten investigated fertilizer treatments (N1, N2, P1, P2, K, N1P1, N1P1K, N2P1K, N2P2, and Control, see Table 1 for details; 5 separate marks per code: 5 different pots). Plant traits abbreviations: BraNo – branched shape of nodules, CylNo – cylindrical shape of nodules, DiRN – diameter of root neck, FanNo – fan-like shape of nodules, GreNo – green color of nodules, LeNod – length of nodules, LeTR – length of taproot, MaxOr – maximal order of branching, NrNod – number of nodules, PinNo – pink color nodules, WhiNo – white color of nodules, WiNod – width of nodules.

Underground organs

There was no significant effect of treatment on length of the taproot, which ranged from 5 to 6 cm in control and N2 treatment, and up to 9 and 12 cm in P2 and P1 treatments, respectively (Table 2, Electron. Appendix 3). Diameter of the root neck and maximal order of the branching were significantly affected by the treatment.

The number of nodules per plant ranged from one in N2 treatment up to 13 in P1 and P2 treatments, and the effect of treatment was significant (Table 2). The length and width of nodules were low in the control, in K treatment, and in all treatments with N application. It was high in P1 and P2 treatments. The effect of treatment on length and width of nodules was significant (Table 2). Cylindrical nodules were most commonly recorded in all treatments. In N2 and control treatments, only 20% and 40% of plants, respectively, had nodules and all nodules were cylindrical. A low proportion of plants with nodules in the control and N2 treatment highly contrasted with all plants with nodules in P1, P2 and N1P1 treatments. Further, only cylindrical nodules were recorded in N1, K, N2P1K and N2P2K treatments. All three types of nodules (cylindrical, branched and fan-like shaped) were recorded only in P1 and P2 treatments (Table 2). Finally, all three colors of nodules were recorded only in all plants of P1, P2 and N1P1K treatments. The most active nodules, as indicated by their pink color, were found in treatments N1, P1, P2, K, N1P1 and N1P1K.

Differences in underground organs among treatments and variability among individual pots within one treatment are well visible from the PCA ordination diagram in Fig. 4. The first axis of the PCA explained 38.5% and all axes 72.6% of the data variability. Similar to aboveground organs, three groups of treatments can be recognized according to their position in relation to the first ordination axis. The first group, on the left side of the diagram, is composed of the control and all treatments with N2 application, the second group, in the center of the diagram, is composed of K treatment and all treatments with N1 application, and the third group, on the right side of the diagram, comprises the P1 and P2 treatments. In the N1P1 treatment, the negative effects induced by N fertilization were reduced.

Almost all recorded below-ground traits were positively correlated, as in the diagram angles between their vectors are acute and directed into the same, right side of the diagram.

Discussion

Seedling emergence

Results of this study indicate poor emergence of *T. arvense* without any additional supply of nutrients and in the case of high mineral N supply when sown into an alkaline soil. Addition of mineral P without any other nutrients increased field emergence of *T. arvense* in comparison with the control three times in the May cohort and five times in the July cohort. Such a huge increase in emergence rate after addition of mineral P, together with the violet rather than green color of seedlings in the treatments without P, indicate strong growth limitation of seedlings by insufficient P supply in alkaline soils. One of the mechanisms leading to an acidicole behavior of *T. arvense* can be therefore traced back to their inability under a high soil pH to acquire enough P for their growth.

Survival of plants

Small seedlings of *T. arvense* were highly sensitive to mechanical damage caused by heavy rains, as almost all seedlings died after heavy rains at the end of May and only several taller plants in P1 and P2 treatments survived. Therefore the rapid early growth is necessary to escape from the apparently highly sensitive seedling stage and to survive up to the autumn, but the rapid growth was recorded only in P1 and P2 treatments. Some seeds seeded in July germinated with a long delay, since the highest number of plants was recorded in October rather than in August. Such delay in germination is a typical feature for legumes with characteristic hard-coat dormancy (Grime, 2001). The high delay in germination of seeds and emergence of seedlings in the N2 treatment was probably caused by negative effects of high mineral N supply on seed germination, as was previously recorded for several other species (Křišťálová et al., 2011; Mandák and Pyšek, 2001) and because of P deficiency in seedlings. High mortality of plants between early August and October was recorded especially in NPK treatments. This was probably because of toxic effects of mineral N on *T. arvense* seedlings, as the species is usually growing under N poor conditions (Ellenberg et al., 1992). We can conclude that P limitation of seedling growth together with high N supply increase mortality of *T. arvense* in alkaline soil.

Aboveground organs

In all treatments without P supply, the plants were unable to continue developing beyond the seedling stage not only after the first seeding in May, but also after the second seeding in July. This may have been caused by the strong P limitation. Further, absolutely no flowering was recorded and thus no seeds were produced in any fertilizer treatment in 2010, but only in treatments with P application in 2011. This is in accordance with results by Tyler (1996) for several other acidicole species which could develop beyond the seedling stage only with additional P supply in alkaline soil. Flowering and seed production was recorded only in the second year, indicating the biennial character of the investigated population. The next explanation of no flowering in the first year can be long-day character of the species as was recorded for several other clover species (Nunes and Ray Smith, 2003). Probably late seeding in July prevented flowering as the species can behave as annual or biannual depending on time of seeding during the vegetation season. In other legumes, flowering decreased linearly with delay of sowing (Karaguzel et al., 2005). Whether this is true also for *T. arvense* requires further research.

Addition of P stimulated development of leaves and therefore positively affected the size of the leaf rosette, overwintering and flowering, as also showed by Bucciarelli et al. (2006) for *Medicago truncatula*. The survival of annual or biennial species is strictly dependent on sufficient seed production and therefore the positive effect of P addition on plant size and reproduction is crucial for successful finishing of the life cycle.

We detect no toxicity of high P supply, although *T. arvense* is a species typically growing on P poor soils. It seems that P toxicity can be recorded only in some legumes (see Pang et al., 2010) and that this is not a common feature of all legumes developed under low P availability. Further, no toxicity of high P supply on *T. arvense* in an acid soil was recorded by Dodd and Orr (1995).

Underground organs

Fertilizer treatments strongly affected underground organs of *T. arvense*. The relatively large root system of plants in P1 and P2 treatments highly contrasted with the poorly developed root systems in other treatments. Root growth was strongly limited by insufficient P supply in treatments without P application. The positive effect of P availability on the size of the root system was also recorded by other authors for different species (Adams et al., 2002; Dunbabin et al., 2004; Hill et al., 2006; Lynch and Brown, 2001; Pang et al., 2010; Raghothama and Karthikeyan, 2005; Svoboda and Haberle, 2006). The most remarkable was the positive effects of P1 and P2 treatments and negative effects of high N application (N2) on nodulation. The positive effect of high P availability and negative effect of high N availability on nodulation is in accordance with results by other authors for other legume species (Adams et al., 2002; Brauer et al., 2002; Li et al., 2011; Roumet et al., 2008). The inability of *T. arvense* to acquire enough P in the alkaline soil thus negatively affected also its N_2 fixation via symbiotic bacteria. This is consistent with conclusions by Høgh-Jensen et al. (2002) for *T. repens*, that below optimum P supply, there was a decline in N_2 fixation.

Increase in P availability affected not only the number of nodules per plant, but also the shape of nodules as their active area. Therefore also the efficiency to fix N_2 increased after P application. The decrease of nodulation can be the consequence of N fertilization, when N acquisition can be realized by uptake of nitrate and ammonium as well. P availability in the soil is thus responsible for the development of different amounts and shapes of nodules. In addition, the P fertilizer used contained sulphur which promotes nodulation (Varin et al., 2010). The application of sulphate could thus increase whole plant dry mass, root length, and nodule biomass.

Several shapes of nodules on one plant of *T. arvense* were rarely recorded under field conditions with low P availability (Chmelíková and Hejcman, 2012; Kutschera, 1960). Under field conditions, cylindrical nodules absolutely dominated and other shapes of nodules can probably become developed only under high P supply.

Conclusions

The acidicole behavior of *T. arvense* is connected with the inability of seedlings to acquire enough P during their emergence under alkaline soil conditions. In all treatments, *T. arvense* was not able to flower in the seeding year and flowered only in P treatments in the second year. This indicates the biennial character of the species in the case of late seeding and points to a strong P limitation of flowering and seeds production in plants grown on alkaline soil. Without P addition, *T. arvense* was not able to develop beyond the seedling stage with such a growing substrate. Nodulation was positively affected by P application and negatively by N application, indicating high connection between N_2 fixation and P supply of plants. Although *T. arvense* is a typical species for P-poor soils, it does not suffer from P toxicity under high P supply.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.flora.2012.09.005.

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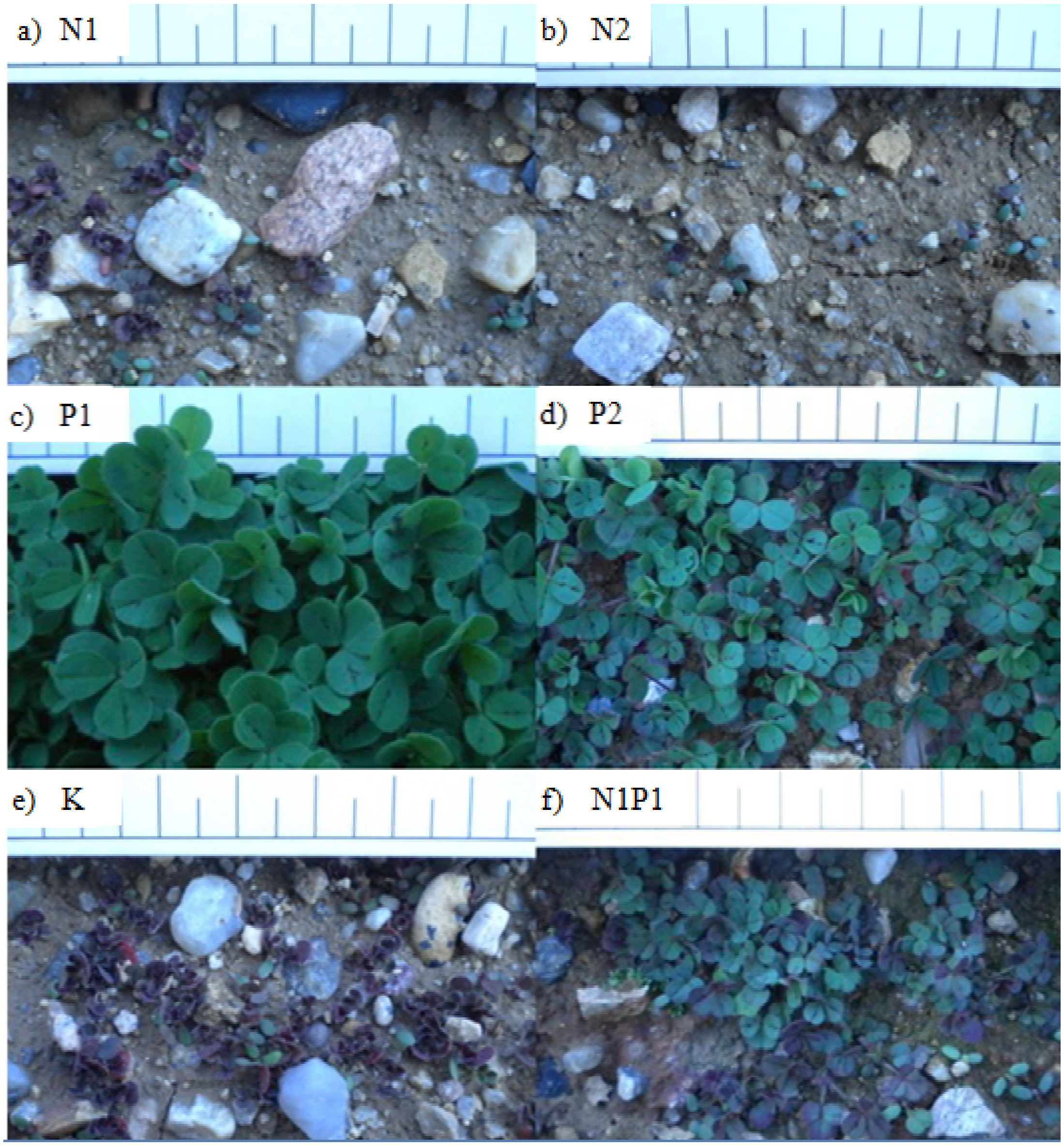
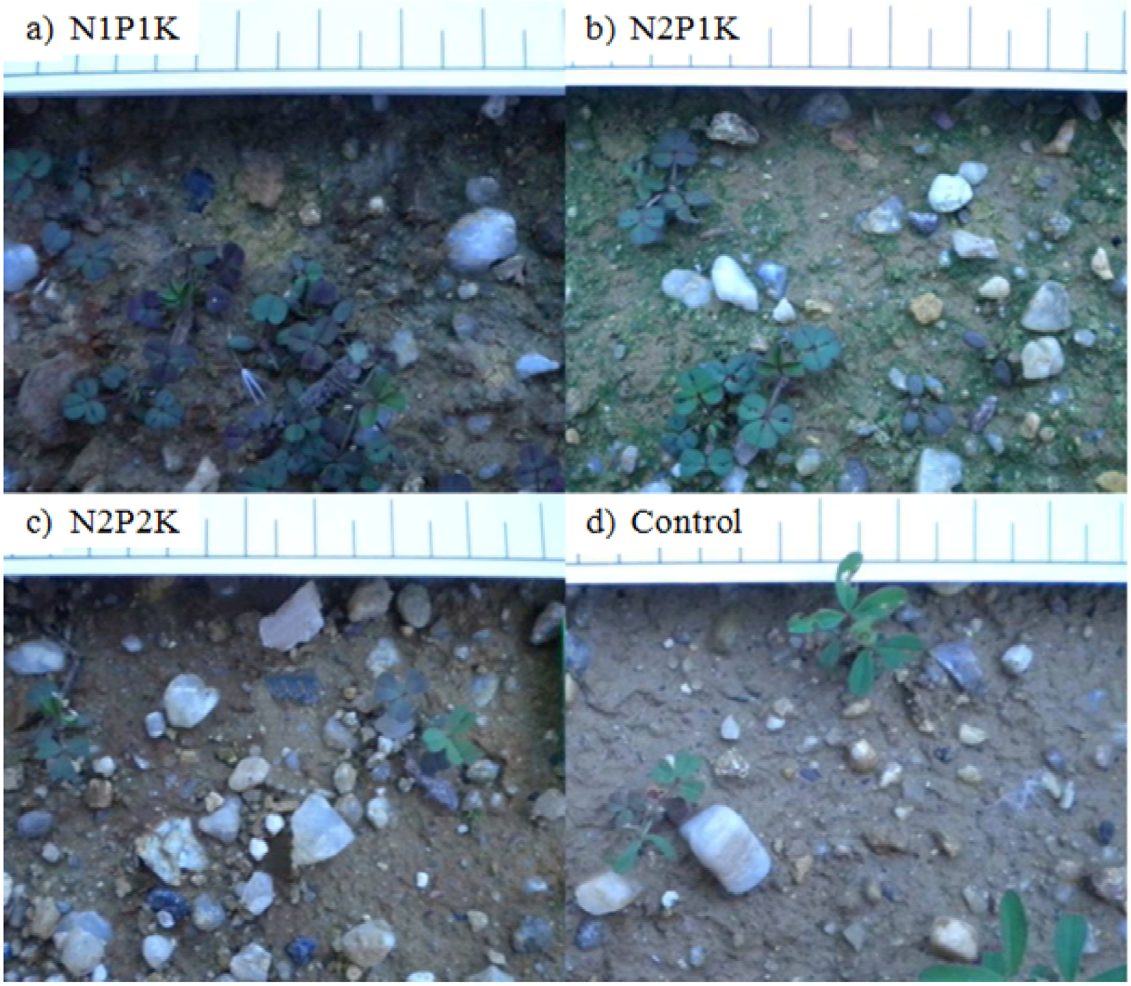
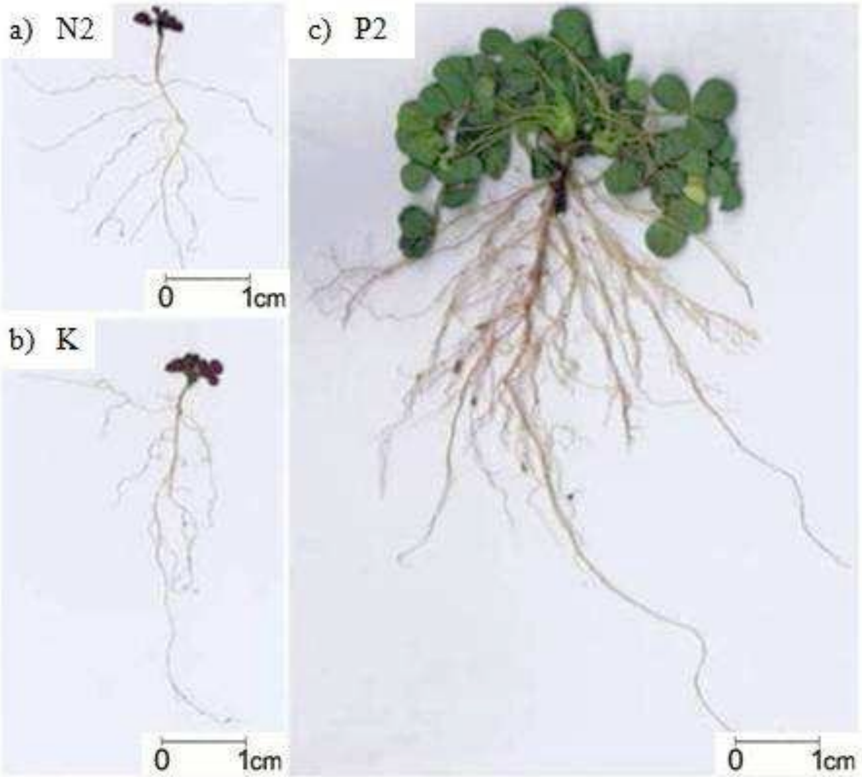
Appendix 1. Effect of (a) N1, (b) N2, (c) P1, (d) P2, (e) K and (f) N1P1 treatments on the aboveground part of plants photographed on 16th September 2010. Treatment abbreviations are given in Table 1.



Appendix 2. Effect of (a) N1P1K, (b) N2P1K, (c) N2P2K and (d) control treatments on the aboveground part of plants photographed on 16th September 2010. Treatment abbreviations are given in Table 1.





Appendix 3. The scanned plants collected in (a) N2, (b) K and (c) P2 treatment on 10th October 2010. Treatment abbreviations are given in Table 1.

III. Effect of nitrogen, phosphorus and potassium availability on emergence, nodulation and growth of *Trifolium medium* L. in alkaline soil

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RESEARCH PAPER

Effect of nitrogen, phosphorus and potassium availability on emergence, nodulation and growth of *Trifolium medium* L. in alkaline soil

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ABSTRACT

Little is known about the effects of nutrient availability on the growth of *Trifolium medium* in alkaline soil. In 2010, a pot experiment (10 N, P and K fertiliser treatments) with seeding of *T. medium* into alkaline soil was performed and emergence of seedlings, survival, aboveground and belowground organs were studied. The positive effects of increased nutrient availability on seedling emergence ranged from 5% in the control to 17% in the high P treatment. The lowest mortality was in treatments with P and K supply and the highest in treatments with N supply, due to the sensitivity of young plants to high N availability. The highest values of most measured aboveground plant traits were recorded in treatments with simultaneous application of N, P and K. There were highly positive effects of P supply alone or in combination with N and K on the development of belowground organs. Taproot length ranged from 11.5 in high N to 40.2 cm in P treatment. There was a negative effect of N application on nodulation, especially in N treatments, where growth of *T. medium* was limited by insufficient P supply. The number of nodules per plant ranged from 0.8 to 4.5 in the high N and P treatments. As demonstrated in this study, *T. medium* is a potentially suitable legume for alkaline soils. It requires a relatively high P and K supply as well as moderate mineral N supply to achieve its maximum growth potential.

INTRODUCTION

Trifolium medium L. (zigzag clover) is a perennial species with flexuous stems up to 50-cm tall, and extensive slender rhizomes, with leaflets commonly 15–40-mm long and petioles up to 8-cm long (Fitter & Peat 1994; Klimešová & de Bello 2009; Chmelíková & Hejcman 2012a). *T. medium* is a typical ecotonal species at the border between tall shrub vegetation and grasslands in Central Europe (Chytrý 2007). The life strategy (*sensu* Grime 2001) is intermediate between SC (stress-tolerant combined with competitor strategy) and CSR (competitor combined with stress-tolerant and ruderal strategy; Hermy *et al.* 1999). According to Ellenberg *et al.* (1992), *T. medium* prefers sun-exposed sites without any specific requirements for soil pH, with low or intermediate nutrient availability and an Ellenberg indicator value for nutrients of 3. *T. medium* has been frequently studied in Australia, particularly because of its potential use for forage production on alkaline soils (Cocks 2001; Dear *et al.* 2007; Suriyagoda *et al.* 2011).

Legumes are well known for their high P and K demand, and low mineral N requirement (Honsová *et al.* 2007; Pavlů *et al.* 2012). The symbiotic relationship of *Rhizobium* bacteria and legumes enables them to fix N_2 from the air, which is inaccessible to many other species (Garg & Geetanjali 2007; Novák 2010). Nitrogen fixation makes legumes highly competitive, especially in environments where plant growth is limited by N availability. Legumes require a relatively high P supply as P availability directly affects N_2 fixation and therefore plant growth (Hellsten & Huss-Danell 2001). Generally, the P requirements of nodulating legumes are higher than those of non-nodulating crops, and the maintenance of adequate P availability in the soil is therefore highly important for high biomass production (Giller & Cadisch 1995; Rochon *et al.* 2004; Erkovan *et al.* 2010; Li *et al.* 2011). In addition to a relatively high P requirement, legumes are also known for their high K, S and Zn requirements (Grewal 2010). An increase in soil pH above neutral is connected with reduced uptake of P, Al, Zn, Cu, Mn and Fe, and with an increased uptake of Ca and Mg in many plant species (Fageria *et al.* 1995; Moreira & Fageria 2010). In addition, decreased K uptake on alkaline soils can be related to antagonistic effects of Ca and Mg (Fageria *et al.* 1995).

Legumes express a wide array of phenotypic traits that improve adaptation to low nutrient availability. Their root system responds markedly more than aboveground organs to different N and P availability in the soil (Johnson & Biondini 2001). Under low nutrient availability, legumes increase biomass allocation to roots, increase the density of the root system and root hairs, create cluster roots or employ mycorrhizas (Adams *et al.* 2002; Denton *et al.* 2006; Sheokand *et al.* 2009; Pang *et al.* 2010; Suriyagoda *et al.* 2010; Wrage *et al.* 2010). Most leguminous species require a neutral or slightly acid soil pH, especially when they depend on symbiotic N_2 fixation (Bordeleau & Prevost 1994). For example, optimal soil pH for alfalfa is pH ($CaCl_2$) 5.4 (Moreira & Fageria 2010). According to Bordeleau & Prevost (1994), soils with a pH ($CaCl_2$) <4.0 or >8.0 are toxic for many legume species and reduce nodulation. In acid and alkaline soils, low P mobility is frequently observed (Baligar 1987). Dodd & Orr (1995), for example, reported P limitation of growth in 18 annual legume species in acid soil.

Although the nutrient requirements of many legume species have been widely studied, little is known concerning the effect of nutrient availability on emergence, nodulation and growth of above- and belowground organs of *T. medium*. Using a pot fertiliser experiment, our aim was to answer the following questions: (i) how is emergence, seedling survival, growth of above- and belowground organs and nodulation of *T. medium* affected by N, P and K availability in alkaline soil; and (ii) is it possible to grow *T. medium* as an alternative legume on strongly alkaline soils (typical for karst areas in the Czech Republic)?

MATERIAL AND METHODS

Experiment description

In April 2010, a pot experiment was established in an open-air vegetation hall in the Crop Research Institute at Prague (Czech Republic, 50°5′ N, 14°18′ E) with natural rain, temperature and light conditions. The experiment was terminated in October 2010 because the plants were removed for analysis of belowground organs. In the pot fertiliser experiment, a combination of two levels of N addition (N1 and N2), two levels of P (P1 and P2) and one level of K (K) addition were used. This resulted in 10 investigated treatments: N1, N2, P1, P2, K, N1P1, N1P1K, N2P1K, N2P2K and a control with no fertiliser input (see Table 1 for details). Each treatment was replicated five times (50 pots in total). The pots were fertilised twice per vegetative season, on 20 April and 27 July, using the following fertilisers dissolved in water: ammonium nitrate with lime ($NH_4NO_3 + CaCO_3$, containing 27.5% N, 10% Ca), super phosphate ($Ca(H_2PO_4)_2 + CaSO_4$, 8.5% P, 20% Ca, 10% S) and potassium chloride (KCl, 50% K, 47% Cl). The pot volume was 30 l and the pot surface area was 1963 cm^2 (pot diameter: 50 cm). Natural soil rich in $CaCO_3$ was used. The soil had the following chemical properties: $N_{total} = 614\ mg{\cdot}kg^{-1}$, $K_{Mehlich\ III} = 62\ mg{\cdot}kg^{-1}$ (low K availability), $P_{Mehlich\ III} = 16\ mg{\cdot}kg^{-1}$ (low P availability), $Mg_{Mehlich\ III} = 422\ mg{\cdot}kg^{-1}$, $Ca_{Mehlich\ III} = 6777\ mg{\cdot}kg^{-1}$ and pH (H_2O) = 9.14. The mean temperature from May to October 2010 was 14.4 °C.

Table 1. Fertiliser treatments and amount of nutrients applied in one dressing. Numbers in parentheses correspond to the total seasonal amount of applied nutrients (two dressings together).

treatment	N ($kg{\cdot}ha^{-1}$)	P ($kg{\cdot}ha^{-1}$)	K ($kg{\cdot}ha^{-1}$)
N1	150 (300)	–	–
N2	300 (600)	–	–
P1	–	40 (80)	–
P2	–	80 (160)	–
K	–	–	100 (200)
N1P1	150 (300)	40 (80)	–
N1P1K	150 (300)	40 (80)	100 (200)
N2P1K	300 (600)	40 (80)	100 (200)
N2P2K	300 (600)	80 (160)	100 (200)
control	–	–	–

Seeds of *T. medium* were obtained from the Research Institute for Fodder Crops, Troubsko, and mother plants used for seed production were collected around Brno town. The seeds were stored at room temperature in paper bags in the dark before the start of the experiment. Fifty seeds of *T. medium* were sown in each pot with pre-fertilised soil on 5 May 2010. The depth of sowing was 1 cm and pots were watered when necessary, to maintain optimal growth conditions during the whole vegetative season.

Seedling emergence

The cumulative number of seedlings (seedling emergence) was recorded up to 22 May, as no further increase in seedling number was recorded after this date.

Number of living plants

The number of living plants was counted in each pot on 21 July, 4 August and 10 October 2010 and expressed as the proportion (percentage) of living plants from the total number of seeds (50) sown per pot. This proportion ensured that the number of plants was directly comparable with seedling emergence recorded in May.

Aboveground organs

On 10 October 2010, the following plant traits were recorded for individual plants of *T. medium* in each pot: (i) plant height; (ii) number of inflorescences; (iii) length of leaf petiole; (iv) length of terminal leaflet; (v) number of stems; (vi) number of leaves per stem; and (vii) number of leaves per plant.

Belowground organs

On 10 October 2010, each plant was carefully removed from each pot and belowground organs were floated in water. Representative plants in each treatment were selected, taking care not to select the smallest or tallest plants. Whole plants were individually digitised using a scanner. Three traits of the root system were recorded: (i) length of the taproot; (ii) diameter of the root neck; and (iii) maximum order of branching. In addition to root characteristics, five traits of nodules were recorded: (i) number of nodules per plant; (ii) width of nodules; (iii) length of nodules; (iv) nodule colour; and (v) nodule shape. The maximum order of branching showed the position of a root within the branched hierarchy of the root system (Eissenstat *et al.* 2000; Wells & Eissenstat 2003). Nodule colour was described as white, pink or green. The proportion of plants with different-coloured nodules was recorded. For example, 100% white nodules indicated that white nodules were found in all plants in a particular treatment. As plants with nodules of all three colours were also frequently recorded, there was a high overlap of colours. The shape of root nodules was classified according to Corby (1971, 1988) as cylindrical or fan-shaped. The proportion of plants with particular nodule shapes from the total number of plants per treatment was recorded. For example, 100% cylindrical nodules indicated that cylindrical nodules were recorded on all plants in a particular treatment.

Statistical analysis

One-way analysis of variance (ANOVA) was used to evaluate the effect of treatment on all collected data. After obtaining significant results, multiple comparisons using Tukey's HSD test were applied, to identify significant differences between treatments. All analyses were performed using STATISTICA version 7.0 (Statsoft, Tulsa, OK, USA). Unconstrained principal components analysis (PCA) was used to visualise similarity between individual pots, treatments and relationships between measured plant traits separately for aboveground and belowground parts. All ordination analyses were performed using CANOCO for Windows version 4.5 and ordination diagrams were constructed with the CANODRAW (ter Braak & Šmilauer 2002).

RESULTS

Seedling emergence

The effect of fertiliser treatment on seedling emergence recorded on 22 May was significant (Fig. 1a). The highest seedling emergence (17%) was recorded in the P2 treatment, followed by the N1 treatment with 16% emergence. The lowest emergence of 6% and 5% was recorded in the N2 treatment and the control, respectively. Emergence in all other treatments was intermediate between these values.

Number of living plants in July, August and October

On 21 July, the proportion of living plants from the total number of seeds sown ranged from 5.6% in the N2 treatment to 13.6% in the K treatment, and the effect of treatment was significant (Fig. 1b). On 4 August (Fig. 1c) and 10 October (Fig. 1d), the plant number decreased and the highest proportion of living plants was recorded in the P1 (9.6% and 10.4%) and K (10.4% and 10%) treatments. The lowest proportion of living plants was in the N2 (1.6% and 1.2%), N2P1K (2.4% and 1.2%) and N2P2K (3.2% and 2.8%) treatments.

Aboveground organs

The effect of treatment on all investigated aboveground traits of plants was significant (Table 2). Generally, the size of plants increased in the order N2, K, control, P1, N1, N1P1, P2,

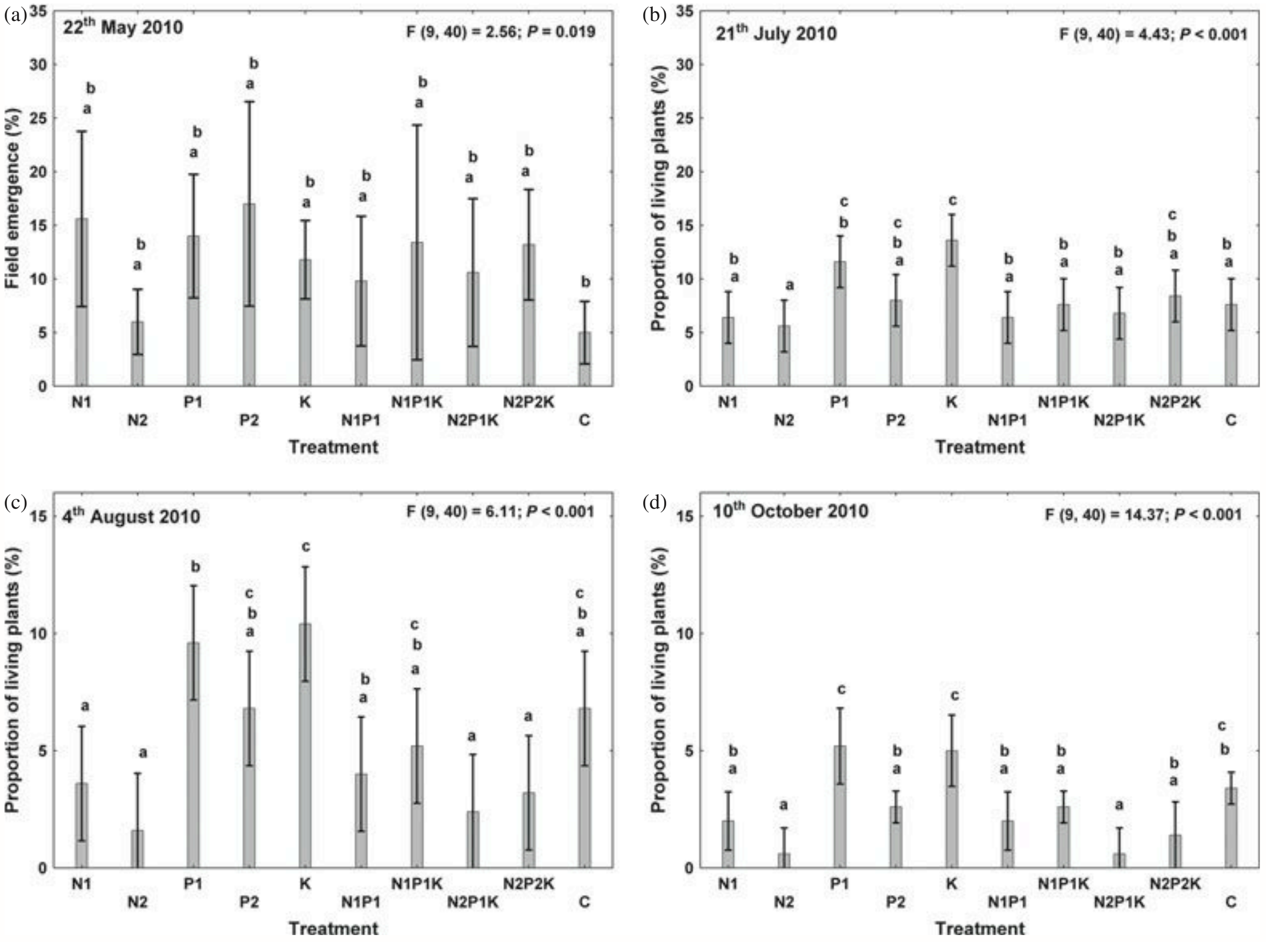

Fig. 1. Effect of investigated fertiliser treatments on (a) seedling emergence of *Trifolium medium* recorded on 22 May, and the proportion of living plants from the total number of seeds sown in each treatment (b) on 21 July, before the second fertilisation, (c) on 4 August, after the second fertilisation and (d) on 10 October. All four (a, b, c and d) figures have the same units and the range of axes and are thus directly comparable. Error bars represent SE of the mean. Treatment abbreviations are given in Table 1. F and *P* values in the upper right corner of each figure represent results of one-way ANOVA. Using Tukey's *post-hoc* test, treatments with the same letter were not significantly different at *P* = 0.05.

Table 2. Summary of aboveground and belowground traits of *Trifolium medium*. Treatment abbreviations are given in Table 1. One-way ANOVA was applied to evaluate the significance of treatment effects.

											one-way ANOVA	
treatment	N1	N2	P1	P2	K	N1P1	N1P1K	N2P1K	N2P2K	m	F	*P*
height of plant (cm)	12.2^{abc}	1.4^{ab}	11.7^{abc}	15.8^{bcd}	3.9^{a}	11.6^{abc}	25.7^{d}	16.7^{abcd}	23.2^{cd}	5.5^{ab}	7.63	<0.001
number of inflorescences	0.3^{ab}	0^{ab}	0.2^{a}	0.3^{ab}	0.2^{a}	0^{a}	1.2^{b}	0.3^{ab}	0.7^{ab}	0^{a}	2.81	0.006
length of leaf petiole (cm)	3.2^{abcd}	0.9^{cd}	3.7^{abc}	3.5^{abd}	1.9^{c}	3.1^{abcd}	4.1^{bc}	4.6^{abe}	5.4^{e}	2.3^{acd}	7.49	<0.001
length of terminal leaflet (cm)	2.2^{abcd}	0.8^{ab}	2.6^{bcde}	3.1^{de}	1.3^{a}	2.2^{abcd}	3.4^{e}	3.4^{cde}	3.9^{e}	1.9^{abc}	9.84	<0.001
number of stems per plant	3^{ab}	1^{ab}	3.1^{ab}	3.2^{ab}	1.1^{a}	3.1^{ab}	4^{b}	3^{ab}	3.1^{ab}	1.3^{a}	3.52	0.001
number of leaves per stem	10.4^{abcd}	4.3^{b}	8.5^{ab}	14.9^{ac}	4.5^{b}	10.5^{abc}	15.2^{ac}	11^{abc}	19.6^{c}	8.3^{ab}	5.99	<0.001
number of leaves per plant	44.9^{ab}	4.3^{ab}	27.9^{ab}	51.3^{ab}	5.5^{a}	47.5^{ab}	67.6^{b}	48.3^{ab}	63.4^{ab}	48.3^{a}	3.20	0.002
length of taproot (cm)	23.1^{bc}	11.5^{c}	40.2^{a}	37.2^{ab}	32.2^{ab}	33.9^{ab}	35.7^{ab}	33.2^{ab}	39.8^{a}	35.3^{ab}	4.63	<0.001
diameter of root neck (cm)	0.5^{abc}	0.2^{a}	0.7^{c}	0.7^{ab}	0.4^{ab}	0.6^{abc}	0.7^{bc}	0.7^{abc}	0.7^{abc}	0.4^{a}	4.25	<0.001
maximum order of branching	2.8^{ab}	2.3^{a}	3.9^{b}	3.2^{ab}	3.2^{ab}	2.6^{a}	2.9^{a}	2.8^{ab}	2.5^{a}	2.5^{a}	5.27	<0.001
number of nodules per plant	0.8^{a}	0.8^{a}	3.4^{c}	4.5^{a}	1.9^{ab}	1.1^{ab}	1.4^{ab}	2.3^{bc}	1.9^{ab}	2.2^{b}	26.02	<0.001
width of nodules (mm)	1^{a}	0.5^{a}	1.7^{a}	1.2^{a}	1.7^{a}	1^{a}	1.2^{a}	1.2^{a}	1^{a}	1.1^{a}	1.51	0.155
length of nodules (mm)	1.2^{a}	0.8^{a}	3.8^{b}	3.4^{b}	3.8^{b}	1.2^{a}	1.9^{ac}	2.0^{abc}	1.4^{a}	3.3^{bc}	9.42	<0.001
white nodules (%)	66^{ab}	75^{ab}	64^{ab}	92^{a}	40^{b}	100^{a}	93^{a}	100^{a}	100^{a}	87^{a}	3.58	0.001
pink nodules (%)	50^{ab}	50^{ab}	100^{a}	100^{a}	100^{a}	71^{ab}	50^{b}	83^{ab}	70^{ab}	100^{a}	4.56	<0.001
green nodules (%)	17^{a}	0^{a}	100^{c}	85^{bc}	73^{bc}	0^{a}	7^{a}	33^{ab}	20^{a}	73^{bc}	11.71	<0.001
cylindrical nodules (%)	17^{a}	75^{ab}	71^{ab}	54^{ab}	73^{ab}	14^{a}	36^{a}	66^{ab}	30^{a}	93^{b}	3.56	0.001
fan-like nodules (%)	0^{a}	0^{a}	21^{a}	38^{a}	20^{a}	13^{a}	14^{a}	0^{a}	0^{a}	7^{a}	1.63	0.118

Using Tukey's *post-hoc* test, treatments with the same letter were not significantly different.

N2P1K, N2P2K and N1P1K treatments. Scanned whole plants and photographs of plants in all studied treatments are provided in Figs 2, 3 and 4. Plant height ranged from <6 cm in N2 and K treatments and the control, to >20 cm in N2P2K and N1P1K treatments. No inflorescences were recorded in N2 and N1P1 treatments or in the control, however >1 inflorescence per plant was recorded in the N1P1K treatment.

Leaf petioles and terminal leaflets <1-cm long were recorded in the N2 treatment and petioles and leaflets >3 cm were recorded in N2P2K, N2P1K and N1P1K treatments. The number of stems per plant ranged from one in N2 to four in N1P1K treatment. The number of leaves per stem ranged from 4.3 in N2 to 19.6 in the N2P2K treatment. The number of leaves per plant ranged from 4.3 in N2 to 67.6 in the N1P1K treatment. Differences among treatments and variability among individual plants within a treatment are clearly visible from the PCA ordination (Fig. 5). The first axis of the PCA explained 70.1% of the variation, and all axes together accounted for 95.5% of data variability.

Belowground organs

With the exception of nodule width and proportion of fan-like nodules, the effect of treatment was significant for all other investigated traits of belowground organs (Table 2). Taproot length ranged from 11.5 to 40.2 cm in N2 and P1 treatments (Fig. 2), respectively. The maximum diameter of the root neck ranged from 0.2 cm in N2 to 0.72 cm in P1 treatment, followed by 0.7 cm in P2, N1P1K, N2P1K and N2P2K treatments. The maximum order of branching ranged from 2.3 in N2 to 3.9 in P1 treatment. The number of nodules per plant ranged from 0.8 in N1 and N2 treatments to 4.5 in P2 treatment. Nodule width ranged from 0.5 to 1.7 mm in N2 and P1 treatment, respectively. Nodule length ranged from 0.8 mm in N2 to 3.8 mm in K and P1 treatments. Except for N2 and N1P1 treatments, nodules of all three colours were recorded in all treatments. Cylindrical nodules were more common in all treatments. In N1P1 and N1 treatments, nodules were only recorded in 14% and 17%, respectively, of plants and all nodules were cylindrical.

Differences in belowground organs among treatments and variability among individual plants within a treatment are clearly visible from the PCA ordination (Fig. 6). The first axis of the PCA explained 30.6% of the variation and all axes together explained 66.3% of data variability. P2 and P1 treatments are clearly separated from all other treatments on the left of the diagram. Treatments with a combination of all nutrients (N1P1K, N2P1K and N2P2K) and the control are located in the centre.

DISCUSSION

Seedling emergence

The number of seedlings indicated poor emergence of *T. medium* in treatments without any additional supply of nutrients or with a high N supply and no other nutrients (N2 treatment) in alkaline soil. In acid soils, Woodman *et al.* (1998) recorded a negative effect of N fertiliser application on seedling emergence of several legume species. In the current study, either no effect or a positive effect of increased nutrient availability on seedling emergence was recorded. This might be related to low nutrient availability in the used soil and therefore to a stimulating effect of increased nutrient supply on early growth of seedlings. A similar positive effect of increased nutrient availability on emergence was recorded in the same soil for *T. arvense* L. (Chmelíková & Hejcman 2012b). This contradicts results for several other species in which a high N supply reduced germination (*Atriplex* spp. Mandák & Pyšek 2001) or seedling emergence (*Rumex obtusifolius* L. and *R. crispus* L.; Křišťálová *et al.* 2011).

Emergence was only partly stimulated by P supply, substantially less than for *T. arvense* (Chmelíková & Hejcman 2012b), *R. obtusifolius* and *R. crispus* (Křišťálová *et al.* 2011), indicating that emergence of *T. medium* is less affected by nutrient availability than in the species with smaller seeds. Seed size, therefore, appears to play an important role in the response of seedling emergence to nutrient availability in the soil. Furthermore, some control seeds germinated with a relatively long delay, as the proportion of living plants on 21 July was higher than on 22 May. A delay in seed germination in the control might be due to slower breaking of the seed coat than in other treatments, as a hard seed coat (physical dormancy) is a typical feature of Fabaceae (Baskin *et al.* 2000).

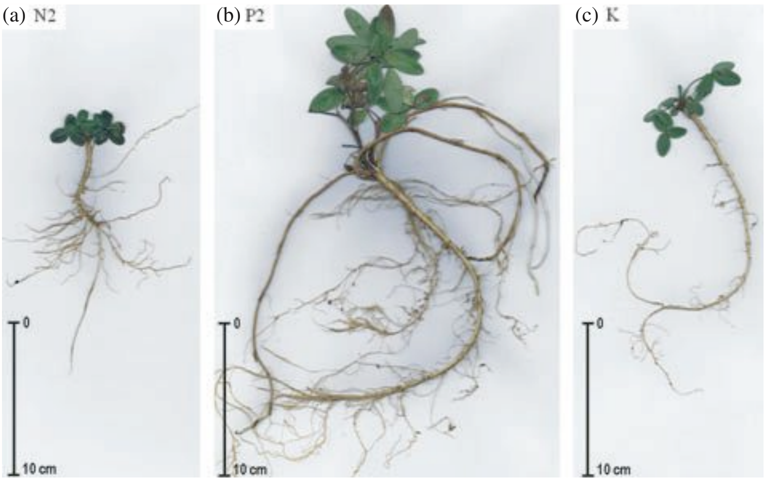

Fig. 2. Scanned plants collected in (a) N2, (b) P2 and (c) K treatments on 10 October 2010. Treatment abbreviations are given in Table 1.

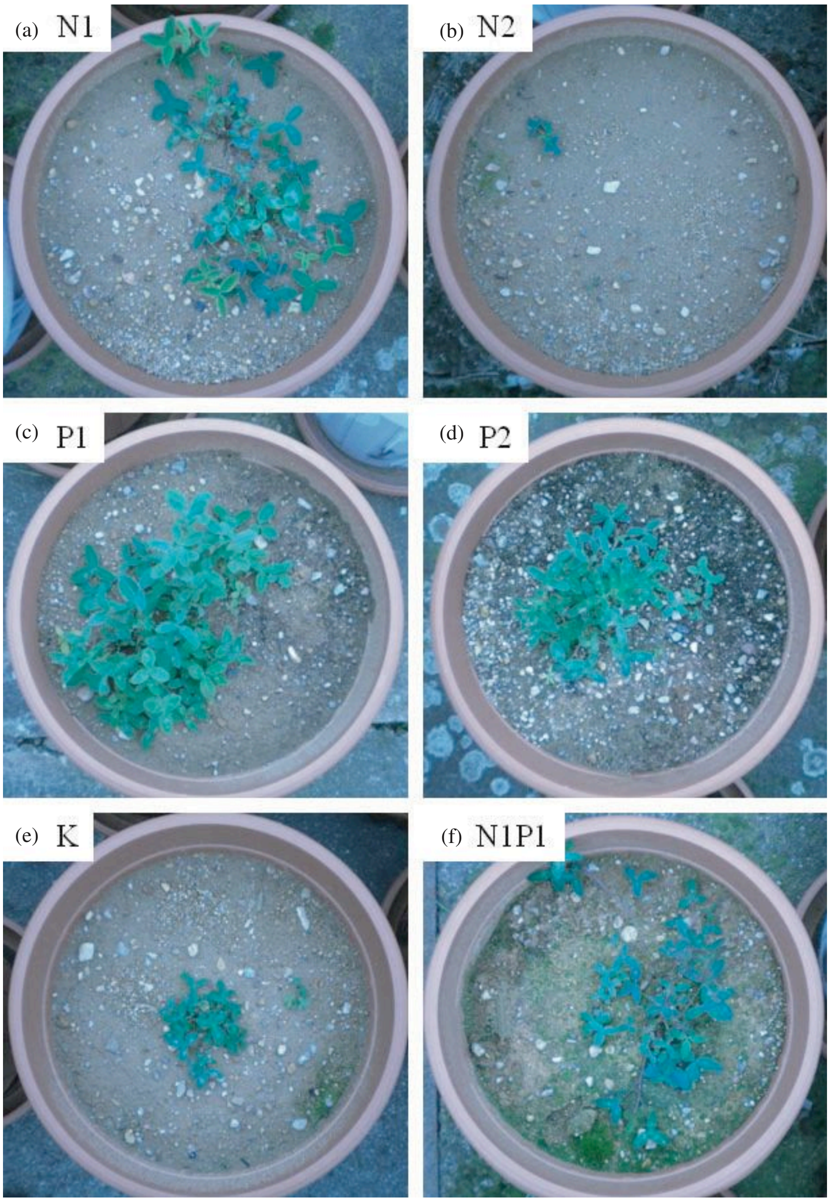

Fig. 3. Effect of (a) N1, (b) N2, (c) P1, (d) P2, (e) K and (f) N1P1 treatments on aboveground part of plants photographed on 16 September 2010 (in a 50-cm diameter and 30 l volume pot). Treatment abbreviations are given in Table 1.

Number of living plants in July, August and October

There was a notably decrease in the number of living plants in treatments with N application between 21 July and 4 August. This was because of the high negative effect of a second N application performed 27 July on individual plants. Following this application, plants in treatments with high N addition withered. This indicates a negative effect of high N supply, especially NH_4^+, on legumes and is consistent with previous results for *T. arvense* (Chmelíková & Hejcman 2012b) and *T. pratense* (Neuberg *et al.* 2011). Legumes are generally substantially more sensitive to high mineral N supply than grasses, and therefore intensive N fertiliser supply reduces the proportion of legumes and benefits grasses in plant communities (Hogh-Jensen & Schjoerring 1997; Britto *et al.* 2001; Honsová *et al.* 2007; Liebisch *et al.* 2013).

The highest survival of plants in P1 and K treatments indicates a positive effect of improved P and K supply on plant survival. An improved K supply generally increases plant resistance to environmental stress (Zahran 1999). A positive effect of P and K application to *T. medium* seedlings is consistent with results for *T. pratense* and *T. repens*, which responded highly positively to P and K application in grassland fertiliser experiments (Honsová *et al.* 2007; Pavlů *et al.* 2012; Liebisch *et al.* 2013). The high P and K requirements of *T. medium* are thus in agreement with the generally high P and K requirements of other grassland legumes.

Aboveground organs

Plant traits with the highest values were recorded in treatments after application of all the tested nutrients. Many studies have demonstrated a positive effect of increased P supply on legumes (Fageria *et al.* 1995; Cruz *et al.* 1997; Patreze & Cordeiro 2005; Bucciarelli *et al.* 2006; Erkovan *et al.* 2010; Pang *et al.* 2010; Suriyagoda *et al.* 2011). A positive effect of P supply on

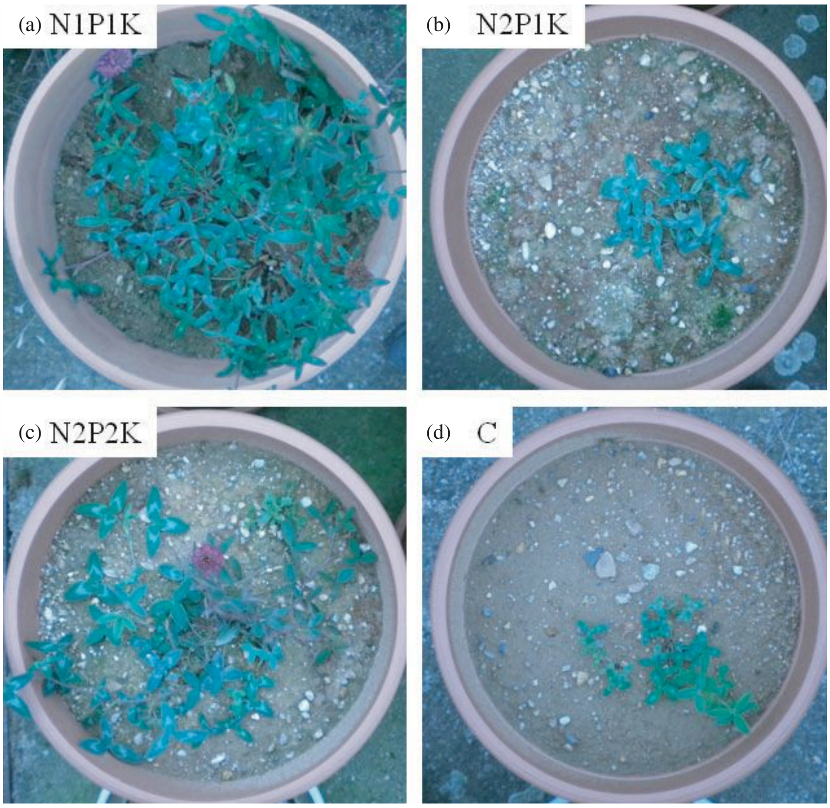

Fig. 4. Effect of (a) N1P1K, (b) N2P1K, (c) N2P2K and (d) control treatments on aboveground plant parts photographed on 16 September 2010 (in a 50-cm diameter and 30 l volume pot). Treatment abbreviations are given in Table 1.

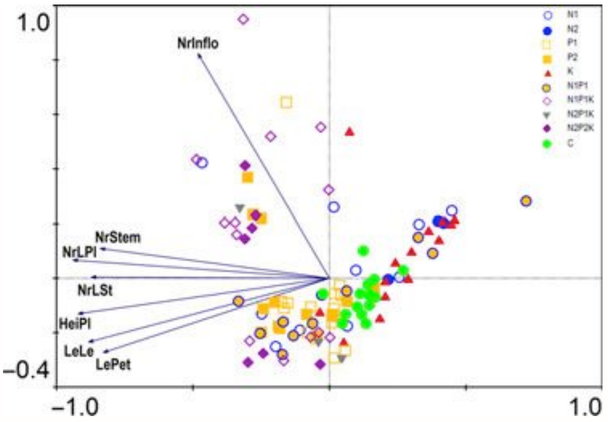

Fig. 5. Ordination diagram showing results of PCA of aboveground plant traits of *T. medium* in 10 investigated fertiliser treatments (N1, N2, P1, P2, K, N1P1, N1P1K, N2P1K, N2P2 and control, see Table 1 for details). Plant trait abbreviations: HeiPl – Height of plant, LeLe – length of terminal leaflet, LePet – length of leaf petiole, NrInflo – number of inflorescences, NrLPl – number of leaves per plant, NrLSt – number of leaves per stem.

growth of aboveground organs was also observed here, but was most positive when P was applied simultaneously with N and K, supporting the findings of Zhao *et al.* (2007). Gates & Wilson (1974) found little effect of K application on aboveground biomass. The tallest plants were in the N1P1K (25.7 cm) and N2P2K (23.2 cm) treatments, indicating relatively high nutrient requirements of *T. medium* not only for P and K, but also for N. On permanent pasture, Ates (2011) observed substantially taller (65.5 cm) plants of *T. medium* than recorded in the current study. Smaller plants in the current experiment were probably related to the short (1 year) duration of the

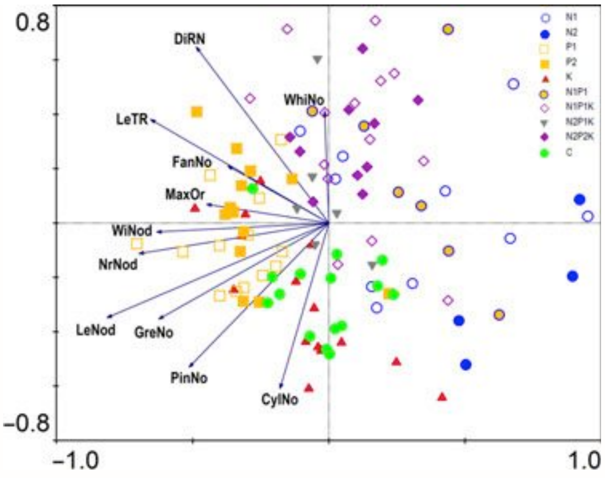

Fig. 6. Ordination diagram showing results of PCA of belowground plant traits of *T. medium* in 10 investigated fertiliser treatments (N1, N2, P1, P2, K, N1P1, N1P1K, N2P1K, N2P2 and control, see Table 1 for details). Plant trait abbreviations: CylNo – cylindrical nodules, DiRN – diameter of root neck, FanNo – fan-shaped nodules, GreNo – green nodules, LeNod – length of nodules, LeTR – length of taproot, MaxOr – maximum order of branching, NrNod – number of nodules, PinNo – pink nodules, WhiNo – white nodules, WiNod – width of nodules.

experiment, as *T. medium* is a perennial species and thus requires several years to achieve its growth potential.

The tall plants of *T. medium* in the N2P2K treatment applied here contrast with very small plants of *T. arvense* found in another N2P2K treatment (Chmelíková & Hejcman 2012b). This indicates substantially higher N requirement of *T. medium* than *T. arvense* and clearly reflects their Ellenberg indicator values for nutrients of 1 for *T. arvense* and 3 for *T. medium* (Ellenberg *et al.* 1992). Although *T. medium* is able to fix N_2 *via* symbiotic bacteria, there is a tendency for it to obtain more mineral N directly from the soil solution when this is available.

There were no inflorescences in N2 and N1P1 treatments or in the control. The positive effect of P addition on flowering was not as large as in the studies of Mabapa *et al.* (2010) and Kumar (2011) with different legume species. In perennial species such as *T. medium*, a highly positive effect of P addition on flowering is usually observed in the second and subsequent years, but not in the first seeding year. A positive effect of P supply on length of the terminal leaflets and petioles is consistent with the results of Bucciarelli *et al.* (2006) and Yahiya *et al.* (1995).

The highest number of stems (four) and highest number of leaves per stem were observed after N1P1K treatment. This is in agreement with results for *T. medium* in pasture vegetation with soil pH 5.9 (Ates 2011). Berg *et al.* (2009) reported similar results for *Medicago sativa*, where addition of P increased forage yield by enhancing biomass per stem. In the current study, the number of stems was low in N2 and K treatments without simultaneous P application, indicating the high P requirement of *T. medium* and that P was a growth-limiting nutrient that directly affects development of different organs.

Underground organs

There was a highly positive effect of P supply on development of underground organs, as also observed following application of all nutrients (N1P1K, N2P1K and N2P2K treatments). There was a negative effect of N application on nodulation, especially in the N1 and N2 treatments, where growth of *T. medium* was limited by insufficient P supply.

The longest taproots of *T. medium* were recorded in treatments with P, in agreement with results for *M. sativa* (Baligar 1987). Application of N considerably reduced rooting depth, as also described in Svoboda & Haberle (2006) for *Triticum aestivum* L. under field conditions. There was an increase in diameter of the root neck in *T. medium* after P treatments and in all treatments with N, P and K application (N1P1K, N2P1K and N2P2K), as also found in experiments of Wahl *et al.* (2001) for non-legume species and Pang *et al.* (2010) for legume species. This positive effect of high P supply on root diameter is consistent with results of Hill *et al.* (2006) for other legume species. The maximum order of branching was found in treatments P1, K and P2, and the minimum in the N2 treatment. Simultaneous N and P application increased root branching similar to the study of Forde & Lorenzo (2001).

The most positive effects of P1 and P2 treatments and negative effects of high N application without other nutrients (N1 and N2 treatments) affected nodulation. A positive effect of P and negative effect of N application on nodulation in legumes is consistent with results of many authors for different legume species (Adams *et al.* 2002; Brauer *et al.* 2002; Roumet *et al.* 2008; Li *et al.* 2011; Chmelíková & Hejcman 2012b). In general, the degree of nodule inhibition after N application depended on the P level, as described in Gates & Wilson (1974) for *Stylosanthes humulis*, where combinations of N and P application stimulated successful nodulation in contrast to separate N application.

CONCLUSIONS

As demonstrated in this study, *T. medium* is potentially a suitable legume species for growth in alkaline soils. It requires a relatively high P and K supply as well as additional mineral N supply to achieve maximum growth potential. The mineral N requirements are substantially higher than for *T. arvense* planted in the same soil (Chmelíková & Hejcman 2012b). Only moderate N supply on soils containing sufficient P and K will benefit growth. High availability of mineral N under low P and K supply can cause high mortality of seedlings, decrease growth rate of aboveground and belowground organs and also decrease the number of nodules per plant.

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IV. Seasonal development of biomass yield in grass-legume mixtures on different soils and development of above- and below-ground organs of *Medicago sativa*

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(submitted)

Článek IV

Seasonal development of biomass yield in grass-legume mixtures on different soils and development of above- and below-ground organs of *Medicago sativa*

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Seasonal development of biomass yield in grass-legume mixtures on different soils and development of above- and below-ground organs of *Medicago sativa*

Abstract

Grass-legume mixtures are suitable for crop rotations under organic farming. Little attention has been paid to seasonal development of mixtures with alfalfa under field conditions. We investigated the effects of site and cut on herbage and below-ground biomass yields and on above- and below-ground traits of *Medicago sativa*. Six sites in southern Germany with alfalfa in mixtures were monitored during the year 2011. Dry matter herbage yield ranged from 9 to 16 t ha^{-1}. The proportion of the first, second and third cuts within the total herbage yield was 45, 36 and 19%. The below-ground biomass in the upper 30 cm soil layer ranged from 1.7 to 3.8 t ha^{-1}.There was no seasonal trend. Diameter of the root neck and maximum order of branching of alfalfa increased during the season. The number of nodules per plant decreased from 9.5–17.0 in May to 7.5–13.0 in August. By the last cut, roots with larger diameter created smaller nodules. More branched roots created more nodules independent of their shape. Thinner roots had more active nodules. Plant height, number of stems and inflorescences per plant were higher in July and August than in May. Mixtures are recommended due to their positive effects.

Keywords: alfalfa; lucerne; nodulation; root traits

Introduction

Alfalfa (syn. lucerne) is one of the most important forage legumes. It is adaptable to a wide range of environments and provides a high forage yield of good quality (El-Din & Assaeed 1995; Berg et al. 2007; Grewal 2010; Moreira & Fagaria 2010). Alfalfa can be planted in monocultures or in grass-legume mixtures, where it increases organic matter content in the soil and also substantially increases N availability via its symbiotic relationship with *Rhizobium* s.l. bacteria. Perennial forage legumes such as alfalfa take a higher proportion of their N from fixation of gaseous N_2 when intercropped with grasses than planted in monocultures. Therefore the use of grass-legume mixtures can result in higher N_2 fixation compared to legume monocultures (Loiseau et al. 2001; Carlsson & Huss-Danell 2003; Nyfeler et al. 2011). Mixtures can thus produce higher herbage yields than the best monocultures and this is called the "overyielding" effect (Halling et al. 2002; Høgh-Jensen & Schjørring 1997; Gierus et al. 2012; Fin et al. 2013). Hence, mixtures play a pivotal role in contributing to the soil nitrogen balance for example in organic farming. In such farming systems maximising the amount of N fixed by plants and retained within the soil is of paramount importance for the yield of the following crop (Hatch 2007).

Root traits are important, because of their close relationship to herbage yield, as the below-ground organs predetermine the production of above-ground organs (Lamb et al. 2000; Annicchiarico 2007). Root traits are heritable. However, they are plastic, to cope with variable soil conditions such as water and nutrient availability (Briggs & Walter 1997; Sultan 2000; Hodge 2004; Šmilauerová & Šmilauer 2010). Roots continually emerge, age, and die throughout the vegetation season at rates that differ among subsets of the root population and change in response to seasonal and environmental factors (Hendrick & Pregitzer 1993; Reid et al. 1993; Forbes et al. 1997; Ruess et al. 1998; Majdi 2001).

Nodulation, which is the colonisation of roots by symbiotic bacteria and the precondition for N fixation, is highly affected by site conditions such as salinity, soil pH, nutrient availability, moisture and temperature (Athar & Johnson 1996; Zahran 1998; Li et al. 2012). For example, a positive effect of high P and a negative effect of high N availability on nodulation were recorded for many legume species (Brauer et al. 2002; Svoboda & Haberle 2006; Roumet et al. 2008; Li et al. 2011). In addition, Shaw et al. (1966) observed a positive effect of molybdenum availability on the number and weight of nodules. Nodulation is also affected by various biotic factors, such as the presence of rhizobia, mycorrhizal fungi, the intensity of photosynthesis and the occurrence of pathogens (Zahran 1998; Guo et al. 2010). According to Corby (1988) nodule shape is associated with legume taxonomy and can be species- or tribe-specific. Cylindrical elongated or fan-like nodules are characteristic for the genus *Medicago*

(Zahran 1998; Chmelíková & Hejcman 2012a). The colour of nodules is a reliable indicator of nodule activity: young and inactive nodules are white; active nodules are pink; moribund or senescent nodules are green, and brown nodules are inactive (Pate & Dart 1961; Swaraj & Bishnoi 1996).

Previous studies (Lamb et al. 2000; Glab 2008; Aranjuelo et al. 2011; Neumann et al. 2011; Testa et al. 2011) have investigated morphological traits of alfalfa roots particularly in monocultures. However, little attention has been paid to nodulation or the seasonal development of nodules if alfalfa is grown in mixtures with other legumes and grasses. The aim of this study was therefore to investigate the seasonal development of root and nodule traits of alfalfa in grass-legume mixtures under field conditions of organic farming. We asked the following research questions: (i) how is the herbage yield and yield of below-ground organs of the grass-legume mixture affected by the locality and time within the vegetation season? (ii) how are the diameter of the root neck, maximum order of branching and nodule traits of alfalfa affected by locality and time? (iii) how are the above-ground plant traits of alfalfa affected by locality and time? (iv) how is the nodule variability in alfalfa affected by locality and time?

Materials and Methods

Study area

All data were collected at six sites (D1, S1, S2, V1, V2, and V3) on organic experimental farms in southern Bavaria (Germany) in the surroundings of Freising (48 24'N, 11 45'O). The altitude of the collection sites (see Table 1 for the description of sites) ranged from 450 to 480 m a.s.l., the mean annual precipitation from 780 to 800 mm and mean annual temperature from 7.8 to 8.4°C. The sites differed in soil conditions, but the climatic conditions (mean annual temperature and precipitation) among sites were uniform due to their spatial proximity. At each experimental site all data were collected in four sampling plots each 10 × 10 m in size. Sample plots were selected in a way to avoid areas with visibly low or high plant density to have a roughly even plant density in all plots. A standard drill technique was used for seeding. Intra-row and inter-row spacing was 12.5 cm. Seeded grass-legume mixtures were homogeneous at all sites and were composed of *Medicago sativa* variety Eugenia (4 kg ha^{-1}), *Trifolium pratense* (5 kg ha^{-1}), *T. repens* (2 kg ha^{-1}), *Dactylis glomerata* (1 kg ha^{-1}), *Festuca rubra* (1 kg ha^{-1}), *F. pratensis* (8 kg ha^{-1}), and *Phleum pratense* (4 kg ha^{-1}). Grass-legume mixtures were established at all sites in 2010, one year before data collection. The exception was the D1 site, where the mixture was established in 2009 and all data were collected in the second year of vegetation. The site D1 is not compared with the other sites because the effect of plant age and the effect of site cannot be separated. Nevertheless, the data from the site D1 was collected in the same way as for the other sites and serve only informatively.

Data collection

Herbage and below-ground organ yield of the grass-legume mixture

Cuts at all sites were performed at the end of May, the beginning of July and at the end of August, according to agronomic requirements and at similar developmental stages. Fresh biomass was measured by cutting the sward mechanically in the central area of each sampling plot (1.8 m × 9.0 m per sample) at a height of about 2 cm. Sub-samples of 0.5 kg were then taken from the cut material and oven-dried at 60°C for 48 h to determine the dry-matter content and herbage yield.

On the day of each cut, soil monoliths 15 cm deep and 7 cm in diameter were collected for the determination of below-ground organ biomass in each sampling plot. Two monoliths (0–15 and 15–30 cm), one from the inter-row space and one from the intra-row space, were collected with an auger. The samples were temporarily stored at -18°C before root separation and counting. Samples were separated using a 0.5 mm mesh sieve. Residues were cleaned with water and then roots were drained in a hydraulic sieving-centrifugation device. Finally, to prevent mould contamination, roots were hand-cleaned from organic debris and immersed in 10% (v/v) ethanol solution.

Below-ground organs of alfalfa

On each day of cutting, five alfalfa plants were selected haphazardly in each sampling plot, taking care not to favour tall or small plants. The monolith method was used to collect the five individual plants (Böhm 1979). The soil monolith measuring 30

× 30 × 30 cm beneath the plant was dug up and washed carefully with water to extract the below-ground organs of the plants from the soil. Subsequently, each whole plant was digitised using a scanner (Brother MFC-6890CDW).

Two traits of the root systems were recorded: 1) diameter of the root neck and 2) maximum order of branching. In addition to root characteristics, four traits of nodules were recorded: 1) the number of nodules per plant; 2) the size of nodules; 3) the shape of nodules and 4) the nodule colour. The maximum branching order reflected the highest developmental branching order in the hierarchy of the root system (Fitter 1987; Eissenstat et al. 2000; Wells & Eissenstat 2003). We therefore refer to roots with no dependent laterals as first order, roots with a single set of dependent laterals as second order, and so on. The shape of root nodules was classified according to Corby (1971; 1988) as cylindrical or branched. We recorded the proportion of plants with the presence of particular nodule shapes from the total number of plants per sampling plot. For example, 100% cylindrical nodules indicated that cylindrical nodules were recorded on all plants in the particular replicate. The colour of nodules was described as pink (active), green (senescent) or brown (moribund) and was expressed as a percentage relative to that of all plants within a treatment. As nodules with two colours were also frequently recorded, there was a high overlap of colours.

Above-ground organs of alfalfa

The above-ground organs were measured at the same five alfalfa plants and were evaluated with roots simultaneously. Five traits of above-ground organs were recorded: 1) plant height – distance between root neck and the tallest point of the plant; 2) the number of stems per plant; 3) the length of the longest terminal leaflet; 4) the length of the longest leaf petiole and 5) the number of inflorescences per plants.

Soil properties

Soil samples were taken in August 2011. In each sampling plot a pooled sample was taken from 0 to 30 cm depth after removing plant residues and roots. Results were averaged to form one representative measure for each site. The soil samples were dried and sieved to 2 mm. All analyses were conducted in accordance with standardised methods of the Association of German Agricultural Analytical and Research Institutes (VDLUFA 1991). Details concerning soil properties at each site together with analytical methods are given in Table 1.

Data analysis

One-way ANOVA was used to evaluate the effects of site and cut on all collected data. After obtaining significant results, multiple comparisons using Tukey's HSD test were applied, to identify significant differences among sites and among cuts. The analyses were based on the values of all measured traits for individual plants. All analyses were performed using STATISTICA 7.0 software (Statsoft, Tulsa). The values of individual yields and plant traits (with exceptions of nodule traits) were compared among five sites (S1, S2, V1, V2, V3). The nodule traits were compared for all sites.

Principal component analysis (PCA) was used to detect trends in the development of above- and below-ground organs during the vegetative season on five sites. Unconstrained PCA analysis was used to visualise similarity between individual plants of five sites, sites and cuts. The relation was visualised separately for above- and below-ground organs of all sites (D1 included). All analyses were performed using the CANOCO for Windows 4.5 program and results of the analyses were visualised in the form of ordination diagrams constructed by CANODRAW program (ter Braak & Šmilauer 2002).

Results

Herbage and below-ground organ yield of the grass-legume mixture

The herbage yield over all three cuts was significantly affected by site and ranged from 8 to 12 t ha^{-1} at D1 and V1 sites, respectively (for details see Table 2). The effect of site on herbage yield of the first cut was not significant, but was significant for the second and third cuts. In addition, the herbage yield was significantly affected by cut at all sites. The proportion of the first, second and third cuts on the total herbage yield over all sites was 45, 36 and 19%, respectively (Table 2).

The biomass of below-ground organs was not significantly affected by site or by time. In the upper 30 cm of the soil, the dry matter biomass of below-ground organs ranged from 1.7 to 3.8 t ha^{-1} in the first cut, from 1.3 to 2.4 t ha^{-1} in the second cut and from 1.7 to 2.8 t ha^{-1} in the third cut (Table 2).

Below-ground organs of alfalfa

The diameter of the root neck, maximum branching order, the number of nodules per plant, nodule size, and the proportion of cylindrical, branched, pink and green nodules were significantly affected by the site. There was no significant effect of site on brown nodules in May. The significant effect of cut on nodule size and on the proportion of pink and green nodules was recorded at all sites (see Table 3 for details).

The largest root diameter was recorded at the S2 site in July. The most branched nodules were recorded in May. The lowest maximum order of branching was recorded at sites S1 and S2 in May, at sites V1 and V2 in July, and at S2 and V1 in August.

With the exception of site S2, the lowest nodule number per plant was recorded in August and the highest in May. The number of nodules per plant ranged from 1.0 at site D1 in July and August to 17.5 at site V1 in May. Cylindrical nodules were the most common at all sites. The proportion of plants with cylindrical nodules ranged from 27% at site D1 to 78–88% at all other sites and the proportion of plants with branched nodules ranged from 8% at site D1 to 72% at site S1. The branched nodules were generally larger than the cylindrical nodules and the size of nodules ranged from 0.8 to 12.4 at sites D1 and V1, respectively. The most active nodules, as indicated by their pink colour, were recorded in May. Afterwards the proportion of plants with pink nodules gradually decreased. The proportion of plants with green nodules was highest at all sites in May and July and the proportion of plants with brown nodules was highest in August.

Differences in below-ground organs among sites and cuts are clearly visible from the PCA ordination diagram in Figure 1. The first axis of the PCA explained 90.1%. The PCA analysis shows, that roots with larger diameter created smaller nodules. This is e.g. well visible in August for the third cut. More branched roots created more nodules independent of their shape. The thinner roots had more active nodules. As a consequence, the most nodules were created in May.

The relatively small change and the low variability in below-ground organs over time at site D1 and their dissimilarity compared to those of other sites is clearly visible in Figure 2a. The D1 points are located in the same quadrant relative to the centre. Higher variability of plant traits was recorded at sites S1, S2, V1, V2, and V3. There was a clear trend in the development of below-ground organs among cuts as is indicated by the shift in the position of marks for individual plots. The plants from the first and the second cut varied more than the plants from the third cut. Differences are so low for the third cut that points of the five sites are located in the same quadrant.

Above-ground organs of alfalfa

We observed a significant effect of site on plant height, the length of the terminal leaflet and leaf petiole (Table 4). The effect of cut was significant for alfalfa plant height at all sites. Generally, the shortest plants were recorded in May. Plant height ranged from 29.6 cm at site S2 in May, to 63.7 cm at site V1 in July (see Table 4 for details).

The largest number of stems per plant (with exception of site D1) was recorded at site V3 (2.4, 2.9 and 5.0). The least number of stems was recorded at site V1 in May and at site S1 in July and August. The shortest leaflet of 1.6 cm and petiole length of 1.8 cm was recorded at site V3 in July. The longest terminal leaflet of 2.6 cm and petiole length of 3.6 cm was recorded at sites S1 and S2 in August. The highest number of inflorescences per plant (3) was recorded at site S2 in July. Only at site D1 inflorescences were recorded in August, too.

Differences in the above-ground plant traits among sites were not as large as for below-ground organ traits. The differences among individual plants during all three months are clearly visible from the PCA ordination diagram in Figure 3. The first axis of the PCA explained 84.2%. In August, the plants from site D1 differed most from plants at other sites (Figure 2b). The plants of the other sites were more similar. This can be seen e.g. in May, where the points of above-ground traits of all five sites are located in the same quadrant.

Discussion

Herbage and below-ground organ yield of the grass-legume mixture

The herbage yield and yield of below-ground organs in the upper 30 cm soil layer was analysed as background information for the detailed research on alfalfa plant traits. The lowest herbage yield of 8 t $ha^{-1}$was recorded at site D1, where both the highest organic matter content and highest N availability in the soil was observed. A possible explanation for this is that the crop mixture at this site was in the second year of vegetation in contrast to the first year of vegetation of mixtures at other sites. Braun et al. (2010) measured a herbage yield of 16 t ha^{-1} when investigating the same mixture in the first year of vegetation at site D1. This gives evidence that site D1 has the highest production potential of all studied sites. A decrease in herbage yield between the first and second year of vegetation was thus 50% and this is roughly in agreement with the decrease of 57–78% recorded between the first and second year of vegetation by Albayrak & Türk (2013). The highest herbage yield of the first and the lowest yield of the third cut agree with the commonly recorded pattern of herbage production in legume stands and grasslands in Central Europe (Hrevušová et al. 2009; Gierus et al. 2012; Hakl et al. 2012a).

Although significant differences in herbage yield were recorded among sites for the second and third cut, differences in below-ground biomass were not significant. Below-ground organ biomass was thus relatively similar at sites with different herbage production, which is in contrast to results of Hakl et al. (2011). Our results thus indicate a shift in favour of roots in the relative proportion of above- and below-ground biomass related to decreasing productivity of sites in terms of total herbage yield.

Luo et al. (1995) recorded higher fine root mass in spring and fall than in summer. This is partly in agreement with the seasonal development of below-ground organs at four of six studied sites (Figure 2). The fine root biomass can be connected with root branching order. The highest mean value of branching order was recorded in the first cut at all five sites.

The legume and grass proportion differed in time as also found by Fin et al. (2013) or Sanderson et al. (2013). Similar to results of Marley et al. (2003) the contribution from different species (e.g. alfalfa and red clover) to total herbage yield differed according to cuts. Stage of maturity affected the yield of alfalfa, but in line with Marley et al. (2003) the influence was not significant in each cut.

Below-ground organs of alfalfa

The differences in root traits were not caused by different climatic conditions at the study sites. Climatic parameters were uniform and the sites differed only in soil conditions. Results showed that root branching was affected by soil conditions and roots with greater diameter developed smaller nodules. Also more branched roots developed more nodules independent of their shape and thinner roots had more active nodules.

In this study, roots at site D1 differed from all other sites. This seems to be caused by the different age of the stand. Root diameter and the number of branched roots were associated with the age of the alfalfa stands, as already shown in other studies (Suzuki 1991; Pietola & Smucker 1995; Hakl et al. 2011).

Root branching was similar at all other sites with one year old plants. The branching order was slightly higher at sites with a higher sand content (V2 and S2), which was probably connected with easier root penetration, similar to the study by Salako et al. (2002). Roots compete with themselves as well. According to Harper (1977) the presence of neighbours affected the environment of a plant and resulted in changes in growth rate or morphology. Hakl et al. (2011) described this effect e.g. for root diameter and number of lateral roots. Small scale differences in stand density at sampled plots together with low number of plants (n=5) led to high variability and unconvincing trends in root traits development over vegetation period. The age of the stand probably had no direct effect on nodule traits, because of their short lifespan between 10 to 12 weeks (Puppo et al. 2005). However, indirect effects can be assumed. Alfalfa plants are known for their thick, long and unbranched roots (Kutschera 1960; Chmelíková & Hejcman 2012a). Older plants of alfalfa have lower number of thin roots, which are more easily infected by bacteria and more likely show nodulation afterwards.

According to many studies (Lynch & Brown 2001; Adams et al. 2002; Dunbabin et al. 2004; Raghothama & Karthikeyan 2005) the effect of nutrient content in the soil seems to be very important. Our data suggested an influence of nutrients on nodules. Unfortunately it is not possible to determine the role of individual nutrients because of their interaction. This is typical for studies under practical field conditions, which are subject to complex environmental and species interactions. At site D1 with the oldest plants, the soil was characterised by a substantially higher N availability than at other sites. This probably explains the low number of nodules, their small size and the low proportion of branched nodules, since nodulation is negatively affected by high N availability in the soil (Adams et al. 2002; Brauer et al. 2002; Beebe et al. 2006; Grewal 2010; Li et al. 2011). Cylindrical and branched nodules can both be observed on roots of alfalfa. However, branched nodules are especially abundant at sites with low N and high P availability, as was observed in our previous study (Chmelíková & Hejcman 2012b). With the exception of site D1, root diameter was slightly higher at sites with a higher availability of soil P in comparison to other localities. This is in agreement with results by Pang et al. (2010) for *M. sativa* in soils with sufficient P availability. Lynch & Brown (2001) and Hill et al. (2006) recorded more branched roots of bean under conditions with low P availability. However, Adams et al. (2002) recorded more branched roots in soils with higher P availability. At the six studied localities, roots were neither more nor less branched and no relation to different P availability was apparent. Differences in P availability among localities were probably not sufficient to be reflected by roots. In addition, there was probably an effect of other nutrients, for example K deficiency at site D1. According to Høgh-Jensen (2006) K deficiency can induce changes in the relative growth of roots and nodules.

In contrast to roots, nutrient availability greatly affected nodules. At site D1 low nodulation in the second year of vegetation due to a high availability of N in the soil can probably explain the substantial decrease in the proportion of legumes and the increase in grasses recorded in grass-legume mixtures in the second and subsequent year of vegetation (Fin et al. 2013). In agreement with results of Zahran (1998) nodule number decreased during the vegetation season at all sites except S2, where a slight increase was observed. The different development at site S2, which has a high content of sand in the soil, might be due to good P availability. The highest number of pink nodules was recorded in May and of green nodules in August. This colour change during the vegetative season was also observed by Swaraj & Bishnoi (1996). There was no positive effect of Mo availability on the number and size of nodules. This contrasts with positive effects of Mo availability on the number and size of nodules on soybean recorded by Vieira (1998) and Toledo et al. (2010). The reason for these different results might be that Mo availability was sufficient at all sites in our study. During the vegetative season, the lowest proportion of brown and green nodules was recorded at the site with higher Mo availability in the soil. This result is in agreement with that of Shaw et al. (1966), reporting the main effect of Mo on nodulation appeared to be a longer period of effective N2 fixation, which leads to more active nodules (pink) than senescent and moribund nodules (green or brown).

Above-ground organs of alfalfa

The highest values for above-ground traits (plant height, the number of stems per plant and leaf size) were recorded for the cut with the highest herbage yield. This is similar to results of other studies (Annicchiarico et al. 2010; Monirifar 2011; Hakl et al. 2012b). According to Sheaffer et al. (2000) the cutting effects determined yield and quality of leaf, stem, and total herbage of six alfalfa entries. Plant height and the number of stems per plant were affected by time of cut within the season. In the second cut, plant height was larger than in the first cut, which agrees with results of Shen et al. (2013) and was probably due to rapid stem growth from the stubble.

Stem number per plant increased with age and was much greater at site D1 with older plants, than at other sites. Many authors (Volenec et al. 1987; Peterson et al. 1992; Sengul 2002) have described the same positive effect of age on alfalfa stem number per plant. According to Singh & Winch (1974) the number of stems increases markedly soon after cutting. With the exception of S1 and S2, the highest stem number per plant was recorded at all sites for the final cut, which agrees with results by Sheaffer et al. (2000). Site S1 and S2 had a quite low pH value not suitable for alfalfa, which prefers pH between 6.5–7.0 (Rhykerd & Overdahl 1972; Peters et al. 2005). Although this difference in soil condition may be one reason for differences in stem numbers, there may be others like small differences in plant density, management, water stress or nutrient availability, which went undetected in this study. The longest terminal leaflet and petiole was measured either in the first or third harvest, indicating some effect of time within the season on leaf shape.

As suggested by Suzuki (1991) the proportion of above-ground alfalfa organs decreased with age and the reduction in older stands was more evident in the second

cut. This is in agreement with our results, as the plant height in the second cut was lower at the site with older plants (D1) in comparison to the other sites.
Inflorescences were only recorded in July (S1, S2, V1, V2, and V3) (at site D1 also in August), which correlates with the flowering time of *M. sativa*. In May, no inflorescences were observed, because the earliest month of flowering for the species is June (Fitter & Peat 1994). More inflorescences were recorded for plants with a higher number of stems. The number of inflorescences seems to be influenced by P content of the soil. However, this effect may be affected by soil pH and clay content, which alter the availability of P to the plants.

Contrasting with results of Wang et al. (2012) we found no dependence of above-ground plant traits on soil type. We suspect that differences in soil physical and chemical properties among investigated sites were probably not high enough in this study.

Stand density or drought stress might be responsible for some of the observed differences in the above-ground plant traits among sites. Drought stress may have occurred at site S2 in spring and might be reflected by shorter terminal leaflets and petioles. The reduction in alfalfa leaflet size and stem weight under drought stress is well known and enables water loss to be reduced (Christian 1977).

Conclusions

This study yielded interesting results on alfalfa in grass-legume mixtures under practical field conditions. Novel findings are thus relevant for agricultural practice and the efficient production of fodder crops.

In the first year of vegetation, the dry matter yield of the herbage ranged from 9 to 16 t ha^{-1} without fertilizer input on common agricultural soils in Bavaria. The proportion of the first, second and third cuts within the total herbage yield was 45, 36 and 19%, respectively. The amount of the dry matter below-ground biomass ranged from 1.3 to 2.8 t ha^{-1} in the upper 30 cm soil layer. There were no clear temporal trends during the vegetation season or significant differences among sites.

In alfalfa plants, the diameter of the root neck and the maximum order of branching generally increased with time and both traits were affected by site conditions. Root branching was slightly higher at sites with a higher sand content, which was probably connected with easier root penetration. A clear seasonal development of nodulation was recorded. The number of nodules per plant generally decreased from 9.5–17.0 in May to 7.5–13.0 in August. At sites with a high availability of N, the number of nodules was only 3.5 in May and 1.0 in August and nodules were very small. By the last cut, roots with larger diameter created smaller nodules. More branched roots created more nodules independent of their shape. Thinner roots, which appear mostly at the beginning of vegetation season, had more active nodules. With the exception of plant height and the number of stems per plants and inflorescences per plant, other above-ground plant traits were minimally affected by time within the season.

Although in this study not all effects and interactions of biotic and abiotic factors could be determined due to the complexity of a practical field study, it was possible to assess above-ground and below-ground aspects simultaneously. Even though not all analysed aspects were significant, our results suggest some important relations between above- and below-ground traits. Future studies should focus on these relations to derive further knowledge that can be used to optimize herbage yields and positive effects of alfalfa and other legume roots.

Finally, we conclude that grass-legume mixtures should be an integral part of crop rotations under organic farming conditions. In such farming systems grass-legume mixtures with alfalfa can provide high herbage yields without fertilizer input and a large amount of below-ground organs that remain in the soil.

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Table 1. Description of study sites.

Table 2. Dry matter herbage yield and yield of below-ground organs in the upper 30 cm of the soil of grass-legume mixtures at sites D1–V3. A detailed description of sites is given in Table 1. P – probability value obtained by the F test. Differences between sites denoted by the same letter (a–c) or differences between cuts denoted by the same letter (A–B) were not significantly different at the 0.05 probability value calculated by *post-hoc* comparisons using the Tukey HSD test.

Table 3. Summary of below-ground traits of alfalfa plants. One-way ANOVA was applied to evaluate the significance of the site effect. Differences between sites denoted by the same letter (a–d) or differences between cuts denoted by the same letter (A–C) were not significantly different at the 0.05 probability value, calculated by post-hoc comparison using the Tukey HSD test.

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Figure 1. Ordination diagram showing the results of PCA of roots and nodule traits of *Medicago sativa* at six sites (S1, S2, V1, V2, and V3– see Table 1 for site description). Plant trait abbreviations: BroNod – brown nodules; CylNod – cylindrical nodules; Diamet – diameter of the root neck; FanNod – fan-like nodules; GreeNod – green

nodules; NodNr – number of nodules per plant; NodSize – size of nodules; OrdBran – maximum order of branching; PinNod - pink nodules.

Figure 2. Principal component analysis (PCA) ordination (a) of below-ground organs and (b) of above-ground organs of *Medicago sativa* on sites D1, S1, S2, V1, V2, and V3 in May (1), July (2) and August (3) 2011.

Figure 3. Ordination diagram showing the results of PCA of above-ground plant traits of *Medicago sativa* at six sites (S1, S2, V1, V2, and V3- see Table 1 for site description). Plant trait abbreviations: Inflor - number of inflorescence; LeLe - length of the terminal leaflet; LeafPet - length of the leaf petiole; NrStem - number of stems per plant; PlHeig - height of the plant.

Table 1. Description of study sites.

	D1	S1	S2	V1	V2	V3
Altitude (m a.s.l.)	450	470	470	480	480	480
Mean annual temperature (°C)	7.8	8.4	8.4	7.8	7.8	7.8
Mean annual precipitation (mm)	800	800	800	790	790	790
Soil type	Stagnic Fluvisol	Haplic Cambisol	Haplic Cambisol	Cambisol	Cambisol	Cambisol
Sand (%)	4	37	43	25	57	18
Silt (%)	71	45	41	58	31	51
Clay (%)	25	18	16	17	12	31
pH[1]	7.3	5.6	5.8	6.3	6.2	7.1
P (mg/100g)[2]	2.6	4.8	6.2	2.2	3.1	7.0
K (mg/100g)[2]	1.7	12.5	10.0	8.3	8.3	10.0
B (mg/kg)[3]	0.3	0.1	0.1	0.3	0.1	0.1
Cu (mg/kg)[3]	4.8	2.9	4.2	3.1	2.2	2.6
Fe (mg/kg)[3]	26	144	181	113	113	94
Mn (mg/kg)[3]	4.7	296	191	314	213	232
Zn (mg/kg)[3]	6.7	9.3	19.4	3.4	3.3	3.9
Mo (mg/kg)[4]	0.11	0.03	0.03	0.03	0.04	0.05
C organic (g/kg)[5]	111.7	9.6	8.8	11.4	11.2	10.2
N total (g/kg)[6]	8.3	1.0	0.8	1.2	1.2	0.9
C : N ratio	9.45	9.6	10.90	9.62	9.64	11.50
N uptake by grass (g)[7]	1.81	0.99	0.84	1.05	1.07	1.03
Age (in years)	2	1	1	1	1	1
Proportion of legumes on herbage yield (%)	60–70	50–70	40–70	60–85	60–75	60–75

[1] measured in a suspension with 0.01 M $CaCl_2$; [2] extracted by a calcium-acetate-lactate solution (CAL); [3] measured by the CAT method; [4] determined by the HWE method; [5] determined by the Dumas dry-combustion method; [7] measured in pot experiment – estimation of N availability in the soil via N uptake by *Lolium perenne* from 10 kg of the soil, extracted for nitrate (0.01 M $CaCl_2$, 1:5 soil-to-extraction ratio), analysed photometrically after separation by HPLC.

Table 2. Dry matter herbage yield and yield of below-ground organs in the upper 30 cm of the soil of grass-legume mixtures at sites D1–V3. A detailed description of sites is given in Table 1. P – probability value obtained by the F test. Differences between sites denoted by the same letter (a–c) or differences between cuts denoted by the same letter (A–B) were not significantly different at the 0.05 probability value calculated by *post-hoc* comparisons using the Tukey HSD test.

		D1	S1	S2	V1	V2	V3	Mean	p
Herbage (t/ha)	May	*3.4*	5.7^{aB}	5.4^{aB}	5.2^{aA}	3.4^{aA}	4.5^{aA}	4.8	0.113
	July	*2.2*	2.9^{aA}	2.5^{aA}	4.8^{bcA}	4.1^{bA}	5.7^{cA}	3.9	**<0.001**
	August	*2.4*	2.4^{bA}	2.3^{bA}	1.9^{abB}	1.4^{aB}	1.5^{aB}	1.9	**<0.001**
	Total yield	*8.0*	11.0^{ab}	10.2^{ab}	11.9^{a}	8.8^{a}	11.7^{a}	10.7	0.134
	P		**0.001**	**<0.001**	**<0.001**	**0.003**	**0.001**		
Below-ground organs (t/ha)	May	*3.8*	2.5^{aA}	1.7^{aA}	2.4^{aA}	2.1^{aA}	2.1^{aA}	2.2	0.586
	July	*2.0*	2.2^{aA}	1.3^{aA}	2.3^{aA}	2.1^{aA}	2.4^{aA}	2.1	0.340
	August	*2.2*	1.9^{aA}	1.7^{aA}	2.2^{aA}	2.8^{aA}	1.7^{aA}	2.1	0.582
	P		0.538	0.318	0.679	0.687	0.847		

Table 3. Summary of below-ground traits of alfalfa plants. One-way ANOVA was applied to evaluate the significance of the site effect. Differences between sites denoted by the same letter (a–d) or differences between cuts denoted by the same letter (A–C) were not significantly different at the 0.05 probability value, calculated by post-hoc comparison using the Tukey HSD test.

Trait	Date	D1	S1	S2	V1	V2	V3	Mean	p
Root neck diameter - Diamet (mm)	May	*8.8*	3.5^{aBC}	3.8^{abBC}	4.2^{abA}	4.8^{bA}	4.4^{abBC}	4.1	**0.005**
	July	*11.8*	6.0^{abAC}	6.9^{bAC}	4.7^{aA}	4.7^{aA}	5.1^{aAC}	6.5	**<0.001**
	August	*10.9*	6.7^{cAB}	6.3^{bcAB}	4.9^{aA}	5.2^{abA}	6.1^{abcAB}	5.8	**0.001**
	MEAN	*10.5*	5.4	5.7	4.6	4.9	5.2		
	P		**<0.001**	**<0.001**	0.165	0.428	**0.001**		
Maximum order of Branching - OrdBran	May	*3.1*	2.5^{aBC}	2.5^{aBC}	3.0^{abBC}	3.0^{abBC}	3.1^{bB}	2.8	**0.001**
	July	*3.2*	2.7^{abAC}	3.2^{bAC}	2.5^{aAC}	2.3^{aAC}	2.6^{abAC}	2.6	**0.001**
	August	*3.6*	2.6^{abAB}	2.3^{aAB}	2.3^{aAB}	3.0^{bB}	2.4^{aAB}	2.5	**<0.001**
	MEAN	*3.3*	2.6	2.7	2.6	2.8	2.7		
	P		0.375	**0.002**	**<0.001**	**<0.001**	**<0.001**		
Number of nodules per plant - NodNr	May	3.5^{cB}	16^{abB}	9.5^{cA}	17.5^{aC}	14.0^{aB}	17.0^{abB}	12.9	**<0.001**
	July	1.0^{aC}	13^{abA}	11.5^{abA}	14.0^{bB}	11.5^{abA}	10.0^{aA}	10.2	**<0.001**
	August	1.0^{aB}	12.5^{aA}	12.0^{aA}	10.5^{aA}	10.5^{aA}	7.5^{aC}	9.0	**<0.001**
	MEAN	1.5	12.5	11.0	14.0	12.0	12.0		
	P	**<0.001**	**0.003**	0.070	**<0.001**	**0.002**	**<0.001**		
Size of nodules - NodSize	May	1.7^{aB}	15.1^{bC}	11.1^{abB}	8.1^{abB}	7.3^{abA}	8.7^{abA}	8.7	**0.009**
	July	0.4^{cA}	8.5^{abB}	3.4^{acA}	12.3^{bA}	6.4^{abcA}	8.7^{abA}	6.6	**<0.001**
	August	0.3^{aA}	8.6^{abA}	9.3^{abA}	16.9^{bA}	7.4^{aB}	3.4^{aB}	7.7	**<0.001**
	MEAN	0.8	10.3	7.9	12.4	7.0	6.9		
	P	**0.001**	**<0.001**	**0.001**	**<0.001**	**0.005**	**0.002**		
Cylindrical nodules - CylNod (%)	May	60^{bB}	100^{aB}	80^{abA}	100^{aA}	90^{aA}	100^{aB}	88	**<0.001**
	July	15^{aB}	90^{aAB}	70^{aA}	95^{aA}	85^{aA}	85^{aAB}	73	**<0.001**
	August	5^{bA}	70^{aA}	85^{aA}	60^{aB}	90^{aA}	60^{aA}	62	**<0.001**
	MEAN	27	87	78	85	88	82		
	P	**<0.001**	**0.016**	0.517	**<0.001**	0.857	**0.003**		
Branched nodules - FanNod (%)	May	10^{aA}	70^{bA}	40^{abA}	25^{aB}	40^{abA}	45^{abA}	38	**<0.001**
	July	0^{bA}	60^{aA}	30^{abAB}	65^{aA}	35^{abA}	35^{abA}	37	**<0.001**
	August	15^{bA}	85^{aA}	80^{aB}	75^{aA}	80^{aB}	65^{aA}	67	**<0.001**
	MEAN	8	72	50	55	52	48		
	P	0.226	0.218	**0.003**	**0.003**	**0.006**	**0.160**		
Pink nodules - PinNod (%)	May	45^{bB}	100^{aB}	80^{aA}	100^{aA}	95^{aA}	85^{aA}	84	**<0.001**
	July	10^{aC}	15^{cA}	60^{aA}	95^{bA}	90^{abA}	85^{abA}	59	**<0.001**
	August	10^{abA}	0^{aA}	0^{aB}	35^{bB}	20^{abB}	10^{abB}	13	**0.005**
	MEAN	22	38	47	77	68	60		
	P	**0.007**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**		
Green nodules - GreeNod (%)	May	45^{bB}	100^{aC}	80^{aB}	100^{aA}	85^{aAB}	95^{aA}	84	**<0.001**
	July	0^{aC}	50^{bB}	35^{bA}	100^{aA}	90^{aB}	85^{aA}	60	**<0.001**
	August	10^{aA}	15^{aA}	20^{bA}	65^{cB}	55^{bcA}	30^{abcB}	33	**<0.001**
	MEAN	18	55	45	88	77	70		
	P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.017**	**<0.001**		
Brown nodules - BroNod (%)	May	15^{aA}	20^{aA}	5^{aB}	0^{aB}	5^{aB}	0^{aA}	8	0.082
	July	0^{bA}	95^{dB}	50^{aA}	20^{abA}	45^{aA}	85^{cdC}	49	**<0.001**
	August	0^{bA}	50^{aA}	75^{aA}	75^{aA}	60^{aA}	40^{abB}	50	**<0.001**
	MEAN	5	55	43	32	37	42		
	P	0.042	**<0.001**	**<0.001**	**0.003**	**0.001**	**<0.001**		

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Trait	Date	D1	S1	S2	V1	V2	V3	Mean	p
Height of the plant - PlHeig (cm)	May	*39.3*	37.2^{abB}	29.6^{cB}	41.1^{bA}	33.8^{acA}	35.7^{abA}	35.4	**<0.001**
	July	*39.8*	46.0^{aA}	51.3^{aA}	63.7^{bB}	49.2^{aB}	54.2^{abB}	52.7	**<0.001**
	August	*47.1*	52.2^{bA}	52.6^{bA}	43.1^{aA}	38.0^{aA}	42.1^{aA}	45.6	**<0.001**
	MEAN	*42.1*	45.1	44.5	49.3	40.3	44.0		
	P		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**		
Number of stems per plant - NrStem	May	*4.4*	2.0^{aA}	2.0^{aA}	1.8^{aA}	2.4^{aA}	2.4^{aA}	2.1	0.233
	July	*3.9*	1.9^{aA}	2.4^{abA}	2.6^{abAB}	2.6^{abAB}	2.9^{bA}	2.5	0.059
	August	*7.1*	1.6^{aA}	1.7^{aA}	3.3^{bB}	3.3^{bB}	5.0^{cB}	3.0	**<0.001**
	MEAN	*5.1*	1.8	2.0	2.6	2.8	3.4		
	P		0.151	0.183	**<0.001**	**0.017**	**<0.001**		
Length of the terminal leaflet - LeLe (cm)	May	*1.9*	2.1^{abA}	1.8^{bB}	2.2^{aA}	2.2^{aB}	2.2^{aA}	2.1	**0.007**
	July	*2.3*	2.3^{aAB}	2.4^{aA}	2.1^{aA}	1.7^{bA}	1.6^{bB}	2.0	**<0.001**
	August	*2.1*	2.4^{aB}	2.6^{aA}	2.4^{aA}	1.8^{bA}	2.0^{bA}	2.2	**<0.001**
	MEAN	*2.1*	2.3	2.3	2.2	1.9	1.9		
	P		**0.029**	**<0.001**	0.103	**<0.001**	**<0.001**		
Length of the leaf Petiole - LeafPet (cm)	May	*3.1*	2.5^{aB}	2.4^{aA}	3.5^{bA}	2.4^{aA}	2.6^{aA}	2.7	**<0.001**
	July	*2.6*	$3.4a^{bA}$	2.3^{abA}	2.2^{abB}	2.4^{abA}	1.8^{aB}	2.4	**0.037**
	August	*2.8*	3.6^{cA}	3.5^{bcB}	2.9^{abcA}	2.7^{abA}	2.6^{aA}	3.1	**0.001**
	MEAN	*2.8*	3.2	2.7	2.9	2.5	2.3		
	P		**0.006**	**<0.001**	**<0.001**	0.841	**<0.001**		
Number of inflorescence per plant - Inflor	May	*0*	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0	n.a.
	July	*0.4*	0.5^{aA}	3.0^{bB}	1.1^{aB}	1.6^{abB}	1.3^{abB}	1.5	**0.002**
	August	*2.8*	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0	n.a.
	MEAN	*1.1*	0.2	1.0	0.4	0.5	0.3		
	P		0.153	**<0.001**	**<0.001**	**<0.001**	**<0.001**		

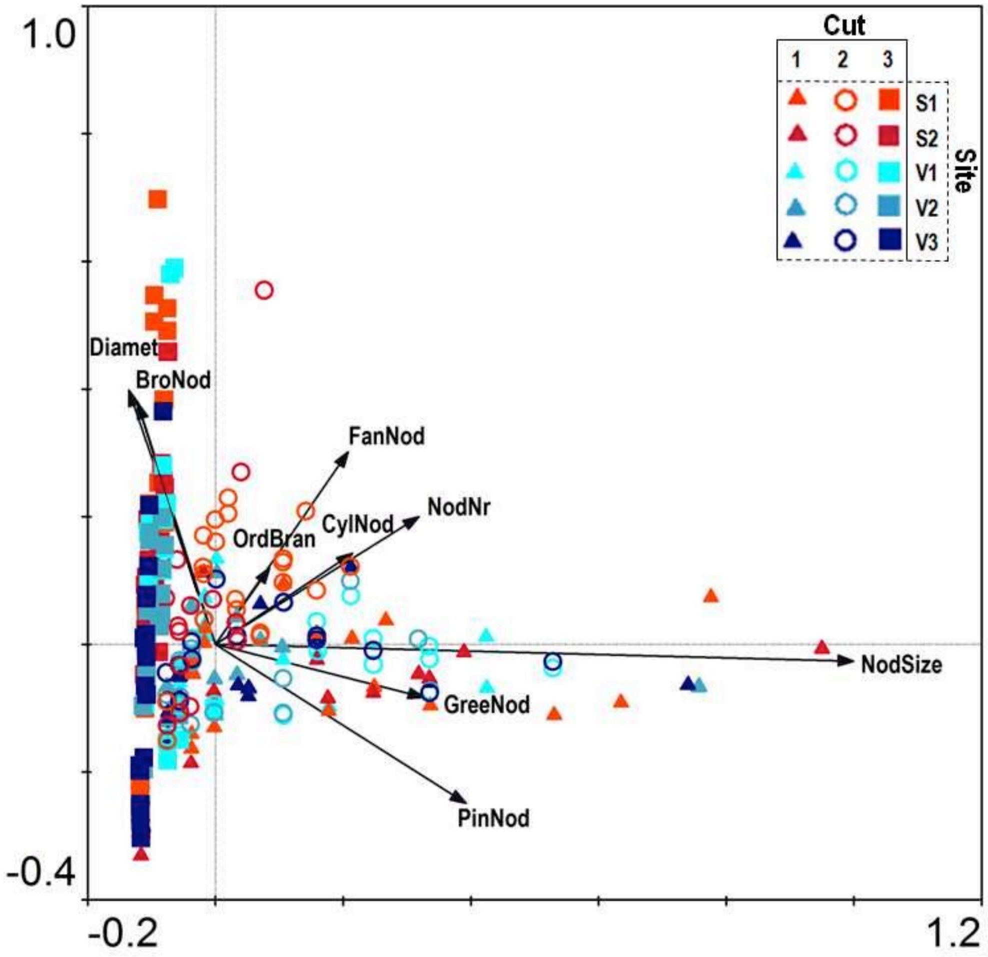

Figure 1. Ordination diagram showing the results of PCA of roots and nodule traits of *Medicago sativa* at six sites (S1, S2, V1, V2, and V3– see Table 1 for site description). Plant trait abbreviations: BroNod – brown nodules; CylNod – cylindrical nodules; Diamet – diameter of the root neck; FanNod – fan-like nodules; GreeNod – green nodules; NodNr – number of nodules per plant; NodSize – size of nodules; OrdBran – maximum order of branching; PinNod - pink nodules.

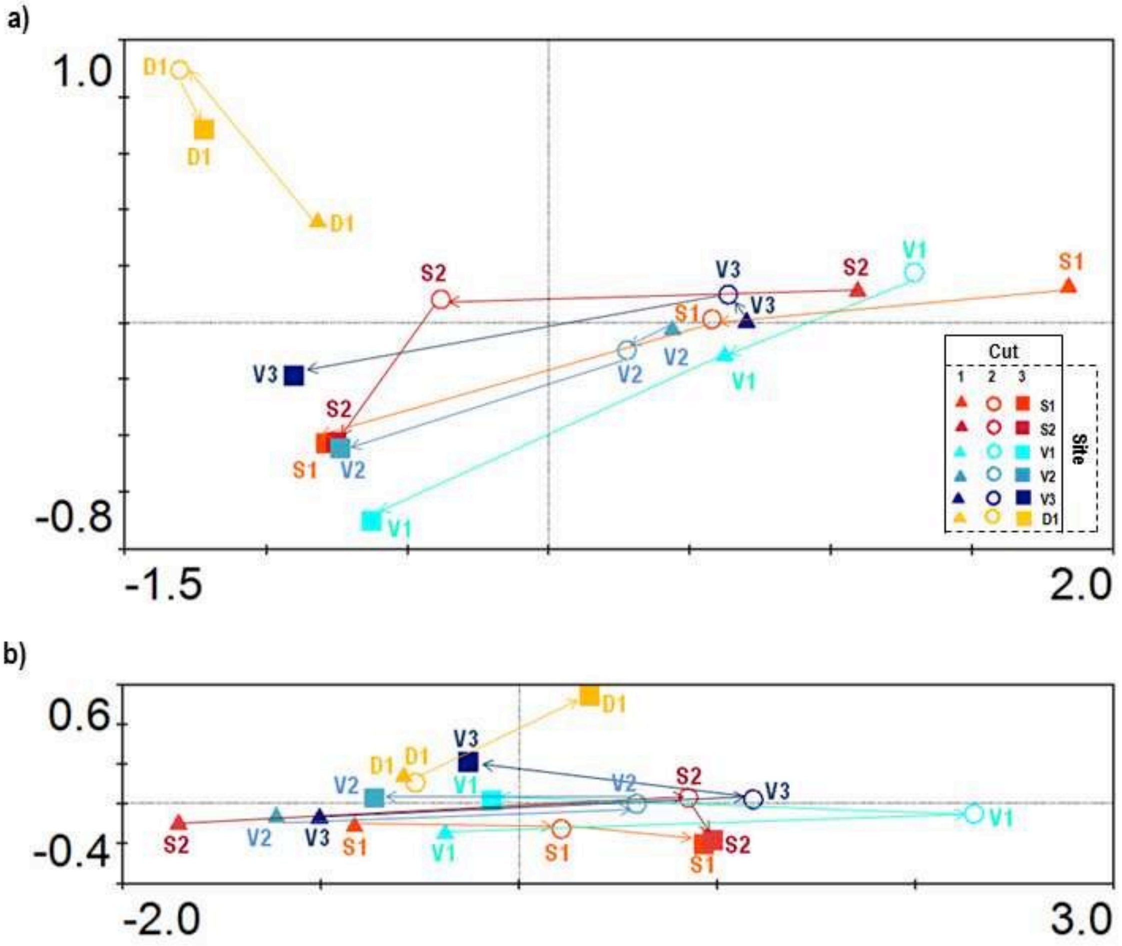

Figure 2. Principal component analysis (PCA) ordination (a) of below-ground organs and (b) of above-ground organs of *Medicago sativa* on sites D1, S1, S2, V1, V2, and V3 in May (1), July (2) and August (3) 2011.

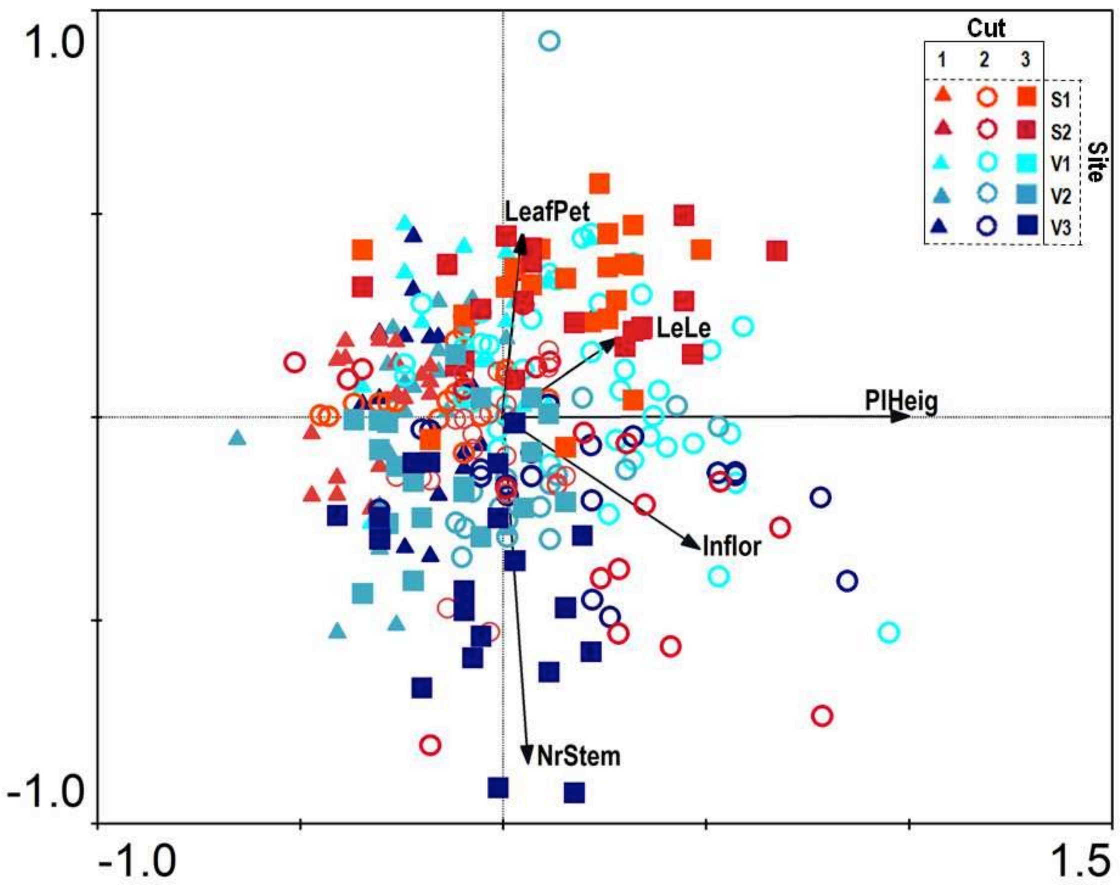

Figure 3. Ordination diagram showing the results of PCA of above-ground plant traits of *Medicago sativa* at six sites (S1, S2, V1, V2, and V3- see Table 1 for site description). Plant trait abbreviations: Inflor - number of inflorescence; LeLe - length of the terminal leaflet; LeafPet - length of the leaf petiole; NrStem - number of stems per plant; PlHeig - height of the plant.

V. Seasonal development of above- and below-ground organs of *Trifolium pratense* in grass-legume mixture on different soils

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Michal Hejcman, Kurt-Jürgen Hülsbergen

(submitted)

Článek V

Seasonal development of above- and below-ground organs of *Trifolium pratense* in grass-legume mixture on different soils

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Key words

Legume; nodulation; red clover; roots; soil texture.

Abstract

Grass-legume mixtures are suitable for crop rotations in organic farming systems. However, little attention has been paid to seasonal development of below-ground organs of *Trifolium pratense* in grass-legume mixtures on different soils. We asked (i) how the above-ground plant traits of red clover are affected by locality and time?, (ii) how the diameter of the root neck, the maximum order of branching and (iii) the nodule traits of red clover are affected by locality and time; Red clover was investigated in grass-legume mixtures in the first year of vegetation on five sites in southern Germany. At all sites sampling took place at the day of cut. Cuts were performed at the end of May, the beginning of July and at the end of August.

The main message of this study is that under similar climatic conditions the root traits (diameter of the root neck, order of root branching, size of nodules, and proportion of senescent nodules) differed according to soil conditions and time within the season. Root diameter increased during the season. Root branching suggested positive influence of sand content. Also more branched roots developed more nodules. Thinner roots had more active nodules (pink color). Usually at the end of season roots develop a bigger diameter and thus have more non active senescent (green color) or moribund (brown color) nodules. Nodule activity differed more according to season than to soil conditions. The number of nodules per plant (12.5 - 19.5) decreased from May to August. Cylindrical nodules were found on 85 - 100% of the plants and branched nodules only on 0 - 25%.

The height of plants was lowest in May and increased in July. The highest mean of number of stems per plant (3.3 - 6.3) was recorded in August. The higher number of plant stems at one site was probably connected with the higher age of these plants.

The results on above- and below-ground traits of red clover and its ability of N_2 fixation provided by this study are not only interesting from a scientific perspective, but also from a practical agronomic point of view. Organic as well as conventional farmers can use these findings in the future to increases benefits from legume crops and improve their overall biomass yields.

Introduction

Red clover (*Trifolium pratense* L.) is one of the most important forage legumes of the temperate zones. The species is known to cope well with a wide range of climatic, soil, pH, environmental and management conditions. It provides good yields even in areas with problems due to soil acidity or excessive soil moisture (*Smith* et al., 1987). Consequently, red clover is an alternative legume species for sites with unsuitable environmental conditions for growing alfalfa. Red clover is cultivated alone or in a mixture with grasses and other legumes (*Abberton* and *Marshall*, 2005; *Hakala* and *Jauhiainen*, 2006). However, it is more frequently sown in mixtures than in monoculture because this leads to higher and more stable dry-matter (DM) yields (*Halling* et al., 2002). Additionally in mixed swards the nitrogen use efficiency is higher (*Scholefield* et al., 2002) and the risk for DM yield loss in case of failure of red clover is less.

Because of the association of legumes with *Rhizobium* bacteria a grass-clover mixture should have a sufficient clover content to optimize the benefits of N_2 fixation and increase the stability of DM yield (*Halling* et al., 2002). Therefore, especially in organic farming it is of high importance for the yield of the following crop to maximize the amount of N_2 fixed and retained within the soil (*Hatch* et al., 2007). Furthermore the deep roots of red clover improve nutrient uptake from deeper soil layers, and provide a high capacity for the uptake of divalent cations like Ca^{2+}or Mg^{2+} (*Frame* et al., 1998). However, soil acidity, which causes phosphorous (P) and molybdenum (Mo) deficiencies, can limit red clover productivity (*Mora* et al., 2005). For that reason specifically Mo plays an important role in legume species due to its involvement in the symbiotic process of N_2 fixation (*Marschner*, 1995; *Ribera* et al., 2010).

T. pratense is a root-splitter meaning that there is normally no vegetative spread unless the root is fragmented due to decay or injury (*Klimešová* and *de Bello*, 2009) Grasses in comparison have their typical fibrous root system. *Fornara* and *Tilman* (2008) found that the complementarity of grass and legume resource use in grasslands increased total biomass and particularly root biomass. This fact leads to greater soil C and N accumulation relative to corresponding monocultures (*Schipanski* and *Drinkwater*, 2012). Thus root traits are important factors influencing DM yield, because the below-ground plant biomass is correlated with above-ground biomass (*Lamb* et al., 2000; *Annicchiarico*, 2007).

In spite of this, there is a lack of studies that investigated the root traits of *T. pratense*. Most studies were mainly on the relationship between above-ground plant traits and yield or between genetic traits and above-ground plant traits but rarely included roots or their nodules (*Riday*, 2008; *Drobná*, 2009; *Grljusic* et al., 2008; *Nikolic* et al., 2010; *Jakešová* et al., 2011; *Pagnotta* et al., 2011; *Tucak* et al., 2013). In addition there is a lack of continuous investigations on changes in above- and below-ground traits of red clover covering an entire growing season. There is a relationship between above- and below-ground plant traits. E.g. *Sturz* et al. (1997) described positive correlation between number of nodules and growth of red clover. Further a correlation was found between growth of adventitious roots and flowering of red clover (*Montpetit* and *Coulman*, 1991).

The aim of this study was to investigate *T. pratense* plant traits in grass-legume mixtures during a complete growing season under field conditions of organic farming. Simultaneously, a study under the same conditions and with the same investigation method was carried out on alfalfa as well (*Chmelíková* et al., submitted). This provides the opportunity to discuss the two most important legume species with respect to their suitability for different soil conditions.

We asked the following research questions for red clover: (i) how are the above-ground plant traits of red clover affected by locality and time, (ii) how are the diameter of the root neck, the maximum order of branching and the nodule traits of red clover affected by locality and time, and (iii) how is the nodule variability in red clover affected by locality and time?

Materials and methods

Study area

All data were collected at six sites (D1, S1, S2, V1, V2, and V3) on organic experimental farms in southern Bavaria (Germany) in the surroundings of Freising (48 24'N, 11 45'O). The altitude of the collection sites (see Table 1 for the description of sites) ranged from 450 to 480 m a.s.l., the mean annual precipitation from 780 to 800 mm and mean annual temperature from 7.8 to 8.4°C. The sites differed in soil conditions, but the climatic conditions (mean annual temperature and precipitation) among sites were uniform due to their spatial proximity. At each experimental site all data were collected in four sampling plots each 10 × 10 m in size. At all sites sampling took place at the day of cut. Cuts were performed at the end of May, the beginning of July and at the end of August.

Sample plots were selected in a way to avoid areas with visibly low or high plant density to have a roughly even plant density in all plots. A standard drill technique was used for seeding. Intra-row and inter-row spacing was 12.5 cm. Seeded grass-legume mixtures were homogeneous at all sites and were composed of *Trifolium pratense* (5 kg ha^{-1}), *Medicago sativa* (4 kg ha^{-1}), *T. repens* (2 kg ha^{-1}), *Dactylis glomerata* (1 kg ha^{-1}), *Festuca rubra* (1 kg ha^{-1}), *F. pratensis* (8 kg ha^{-1}), and *Phleum pratense* (4 kg ha^{-1}). Grass-legume mixtures were established at all sites in 2010, one year before data collection. The exception was site D1, where the mixture was established in 2009 and all data were collected in the second year of vegetation. The site D1 is not compared with the other sites because the effect of plant age and the effect of site cannot be separated. Nevertheless, the data from the site D1 was collected in the same way as for the other sites and is presented for the sake of completeness.

To describe the production potential of each sampling plot, background information on the total yield and the average biomass of below-ground organs in the upper 30 cm soil layer from *Chmelíková* et al. (submitted) were added to Table 1. The three cuts contributed with 45%, 36% and 19% to the total herbage yield. Details of the methods used to determine herbage yield and below-ground biomass are described in *Chmelíková* et al. (submitted).

Data collection

Below-ground organs of red clover

On each sampling date five plants were selected in each sample plot, taking care not to favor tall or small plants. The monolith method was used to collect individual plants (*Böhm*, 1979). The soil monolith measuring 30 × 30 × 30 cm beneath the plant was dug up and washed carefully with water to extract the belowground organs of the plants from the soil. Subsequently, each whole plant was digitized using a scanner.

Two traits of the root systems were recorded: 1) diameter of the root neck, and 2) maximum order of branching. Root neck is also known as the root collar or root crown, which is the part of a root system from which a stem arises. The maximum branching order reflected the highest developmental branching order in the hierarchy of the root system (*Fitter*, 1987; *Eissenstat* et al., 2000; *Wells* and *Eissenstat*, 2003). We therefore refer to roots with no dependent laterals as first order, roots with a single set of dependent laterals as second order, and so on. In addition to root characteristics, four traits of nodules were recorded: 1) the number of nodules per plant, 2) the size of nodules, 3) the shape of nodules, and 4) the nodule color. The shape of root nodules was classified according to *Corby* (1971, 1988) as cylindrical or branched. We recorded the proportion of plants with the presence of particular nodule shapes from the total number of plants per replicate. For example, 100% cylindrical nodules indicated that cylindrical nodules were recorded on all plants in the particular replicate. The color of nodules was described as pink (active), green (senescent) or brown (moribund) and was expressed as a percentage relative to that of all plants within a replicate. As nodules with two colors were also frequently recorded, there was a high overlap of colors.

Above-ground organs of red clover

Five traits of above-ground organs were recorded: 1) the plant height, 2) the number of stems per plant, 3) the length of the terminal leaflet, 4) the length of the leaf petiole, and 5) the number of inflorescences.

Soil properties

Soil samples were taken in August 2011. At each site, four separate subsamples were taken from 0 to 30 cm depth after removing plant residues and roots. Subsamples were combined to form one representative sample. The soil samples were dried and sieved to 2 mm. Standardized methods in accordance with the Association of German Agricultural Analytical and Research Institutes (*VDLUFA*, 1991) were used to measure characteristic soil properties. We choose the results on N uptake by grass from a simple pot experiment growing *Lolium perenne* in soil gathered from the sample sites as a consistent and comparable indicator of N availability to plants. Detailed information on soil properties at each site together with analytical methods is given in Table 1.

Data analysis

One-way ANOVA was used as a univariate method to separately assess differences between the sites and between the cuts during the season for all data collected. After obtaining overall significant results, multiple comparisons using Tukey's HSD test were applied to identify significant differences among sites and among cuts. All analyses were performed using STATISTICA 7.0 software (Statsoft, Tulsa). The plant traits (with exceptions of nodule traits) were compared among five sites (S1, S2, V1, V2, V3). The nodule traits were compared for all sites. Principal component analysis (PCA) was used as a multivariate method to detect more complex trends in the development of above- and below-ground organs during the vegetative season at five sites (S1, S2, V1, V2, V3). Unconstrained PCA analysis was used to visualize similarity between plants at five sites and cuts. Trends were visualized separately for above- and below-ground organs of all sites (D1 included). All analyses were performed using CANOCO for Windows 4.5 and results of the analyses were visualized with ordination diagrams constructed by CANODRAW (*ter Braak* and *Šmilauer*, 2002).

Results

Below-ground organs of red clover

The diameter of the root neck, the maximum order of branching, the size of nodules, and the proportion of brown nodules were significantly affected by the site on all dates. The diameter of the root neck and the number of brown nodules were significantly affected by time at all sites (see Table 2 for details).

With exception of site D1, the largest root diameter was recorded in May and July at site S2 (6.7 and 8.7 mm) and in August at site S1 (9.8 mm). The lowest root diameter was recorded in May at site S1 (5.5 mm), in July at V1 (6 mm) and in August at V3 (6.9 mm). The highest mean maximum order of branching was recorded in May at site S2. The maximum order of branching ranged from 2.3 at site S1 in July to 4.3 at the site S2 (and at site D1 in August as well).

The most nodules were measured in May at all sites (18-19.5), except for the site D1 (12.5). The lowest number of nodules per plant was recorded in May and July at site D1 and in August at site V1 and V3. The biggest nodules were measured in August (D1, S1, S2, and V2) and July (V1 and V3). Cylindrical nodules were found on 85-100% of the plants. On the other hand, the branched nodules were recorded on only 0-25% of the plants. At sites D1, S1, S2 and V1 the branched nodules were found in August. In July they were only recorded at site V1, whereas in May at site V2 and V3. The branched nodules were always bigger than the cylindrical.

High N_2 fixation activity of nodules, indicated by their pink color, was recorded in May for 99% of nodules. The amount of pink nodules decreased with time to 80% in July and 37% in August. The nodules with the longest activity period were recorded in August on site D1. Some pink nodules were partially green. The majority of green nodules (100% of plants) was recorded in May at sites S1, S2 and V3 as well as in July at the sites V1, V2 and V3. The majority of green nodules on site D1 was recorded in May. The least brown nodules (0-10%) were recorded on the sites S1, S2, V1, V2, and V3 in May. At site D1 the least brown nodules (5%) were found in July. The mean proportion of brown nodules increased in time- from 13% in May to 76% in July.

Differences in belowground organs among sites and variability among individual plants in all three months are visible from the PCA ordination diagram in Figure 1. The first axis of the PCA explained 55.6% of the variability in the data. In May the plants had the lowest variability in comparison to July and August. In Fig. 1 it is visible, that the plant traits differed according to cut. During the season plants increased their root diameter and the nodules became moribund (brown). More branched roots created more nodules. The nodules had mostly cylindrical shape and only rarely fan-like shape.

The differences in the development over the season are shown in Figure 2a. Different trends for the development were observed at site D1 (especially the first and second cut) and V3 (especially the second and third cut).

Above-ground organs of red clover

A significant effect of site was found for all above-ground traits except number of stems per plant in May, length of the terminal leaflet in August and number of inflorescences in May and in July (see Table 3).

Only at site V3 no significant effect of season was found for number of stems per plant, length of the terminal leaflet and for the length of the leaf petiole.

Plant height ranged from 29.0 cm at site V2 in May to 70.6 cm at site V1 in July. Generally, the height of plants was the lowest in May and increased in July. In August plant height was high at the site S1 and S2 as well. Excluding D1 the highest number of stems per plant was measured at site V1 in May (3.7), at S2 in July (4.5) and at S1 in August (6.3). In May, the lowest number of stems was recorded at site S1. At site V1, the lowest number of stems was measured in July and August. Finally, the highest mean number of stems per plant was recorded in August.

The longest terminal leaflet (4.8 cm) was recorded at site S2 in August and the longest leaf petiole (31.9 cm) at site S1 in July. The shortest terminal leaflet was found at site V2 in May. The shortest length of the leaf petiole was measured at site S2 in May as well. The mean length of the terminal leaflet and the leaf petiole increased in time.

In May no inflorescences were recorded at sites V1, V2 and V3. No inflorescences were found at sites V2 and V3 in August as well. In July, inflorescences were found on each site. The most inflorescences per plant (1.8) were recorded at site S1. At site D1 2.7 inflorescences were recorded in July.

The differences among individual plants in all three months are visible from the PCA ordination diagram in Figure 3. The first axis of the PCA explained 84.2%. The differences between the sites S1 and S2 as well as the sites V1, V2 and V3 for the second and third cut are shown. The highest values of above-ground plant traits were recorded at sites S1 and S2 for the third and second cut simultaneously. At the sites V1, V2, V3 the highest values were recorded in the second cut. The similar development in time is well visible in Fig. 2b. The trend in development of site D1 was similar to sites V1, V2 and V3, but there is a difference for the second cut.

Discussion

Below-ground organs of red clover

Clearly the main result of this study is that under similar climatic conditions the root traits differed not only according to soil conditions but during the season as well. Root diameter e.g. increased during the season. Root branching suggested positive influence of sand content. More branched roots developed more nodules. Thinner roots had more active nodules (pink color). Roots with bigger diameter, which develop normally at the end of the season, had non active senescent (green color) and moribund (brown color) nodules. Nodule activity differed more according to season than to soil conditions.

Roots at site D1 differed from all other sites may be due to the different age of the stand. On average root diameter of red clover plants was higher at site D1. Such an association of root traits with the age of stands was already shown for alfalfa in other studies (*Suzuki* 1991; *Pietola* and *Smucker* 1995; *Hakl* et al. 2011). Nonetheless the effect could not be separated from the site conditions. Nevertheless the difference between one and two year old plants of red clover seems to be much lower in comparison to differences found for *M. sativa* (*Chmelíková* et al., submitted). The age of plants positively influenced root diameter and maximum order of branching as found by many other authors (*Suzuki*, 1991; *Pietola* and *Smucker*, 1995; *Hakl* et al., 2011). Generally, the roots of red clover were more branched in comparison with the results on roots of alfalfa from our previous study. For red clover there was no relationship between branching and time within season. Contrary to red clover the branching of alfalfa increased with time (*Chmelíková* et al., submitted). The branching order was a bit higher at sites with higher sand content (V2 and S2). *Salako* et al. (2002) suggested that roots will branch more at sites with better possibility of penetration e.g. with high sand content as at sites V2 and S2. *Archer* et al. (2002) and *Glab* (2008) found decreased root volume with decreasing sand content. However, the lowest sand content, which occurred at site V3, had no effect on root volume. Root diameter at V3 was not larger than at other sites. The possibility of penetration into the soil by roots is affected by plowing. At plowing depth, which was visible in the soil profile, considerable differences in the thickness of tap roots were observed at all sites with plowing management.

Observed tap roots of red clover had cylindrical nodules, as described by many other authors (*Corby*, 1971; *Sturz et al.*, 1997; *Klimešová* and *de Bello*, 2009; *Chmelíková* and *Hejcman*, 2012a). Our results suggested a slightly positive effect of sand content in soil (site S2 and V2) and a slightly negative effect of clay content (D1 and V3) on root diameter. In comparison to clay soils Mela (2003) showed for well aerated sandy soils, that red clover developed deep and strong roots and nodules. *Pardo* et al. (2000) observed a quite homogeneous distribution of roots in fine soil. The spatial distribution of roots and the ability of plants to take up water were strongly affected by soil structural conditions. According to *Jansen* et al. (2010) soil type explained 42% of variation in water uptake by lupine. However, the strengths of this relation seem to be dependent on the species. Thus *French* (2002) observed the same for pea, but results were not significant.

According to *Puppo* et al. (2005) nodules have a short lifespan of only 10-12 weeks, meaning that the age of plants has no direct influence on nodule traits. *Voisin* et al. (2002) and *Hatch* et al. (2007) observed nodulation and found a tendency of decreasing N_2 fixation activity with legume age. This was also shown by our results on nodulation at the site D1 with two year old plants. Red clover created more nodules than alfalfa (*Chmelíková* et al., submitted) at the same site with high nitrogen availability. The nodules of red clover were much smaller than the ones of alfalfa. Simultaneously, according to *Jensen* (1986), *Voisin* et al. (2002) and *Cupina* et al. (2010) N_2 fixation activity depended on growth rate and phenology during the growth period and was generally decreasing with flowering and maturation. As described by these authors we found that when inflorescence increased the number of nodules decreased. At five out of six sites the nodule activity (proportion of pink nodules) decreased during the season. Only at site D1 the proportion of active nodules was high during the whole season. This exception can be explained by higher content of molybdenum in the soil. Many authors (*Marschner* 1995, *Leite* et al., 2009; *Toledo* et al., 2010; *Farooq*, 2012) found molybdenum to increase the number and the weight of nodules and thus improving the symbiotic process of N_2 fixation of legumes. The importance of molybdenum is also pointed out by results of *Shaw* et al. (1966) on alfalfa. This study reported that the main effect of Mo on nodulation appeared to be a longer period of effective N_2 fixation, which means that over time more nodules stayed active (pink) and did not become senescent or moribund (green or brown). The same influence on activity of nodules was recorded at site D1 for alfalfa as well (*Chmelíková* et al., submitted).

Furthermore, the negative influence of high amounts of mineral nitrogen in the soil on nodulation was recorded by many authors (*Buttery* et al., 1992; *Voisin* et al., 2002; *Peoples* and *Griffiths*, 2009). Simultaneously mineral nitrogen enhances plant and thus root growth. Both impacts were probably found at site D1. In contrast, the positive influence of molybdenum and potassium on the nodulation of clover (*T. alexandrinum*) described by *Xia* and *Xiong* (1991) and Gates and *Wilson* (1974) could not be proved.

Different amounts of roots in the subsoil may also influence the relationship between acid-soluble potassium (K) and K uptake from soil reserves, because the exploited soil volume thereby varies. Generally, grass roots are concentrated near the surface and are thinner and more finely branched than the roots of red clover (*Evans* 1977; *Sveistrup* and *Haraldsen*, 1997). In contrast, clover roots spread out deeper and the amount of clover may therefore influence K uptake from the subsoil (*Ogaard* and *Hansen*, 2010). The differences could be an advantage of clover concerning the uptake of immobile nutrients. The different soil reactions (pH between 5.6 and 7.3) did not affect the nodulation. Opposed to that, *Brauer* et al. (2002) found increased numbers of nodules in soil with higher soil reaction.

At the sites with higher numbers of nodules, the yield was higher as well. There was a positive relationship between nodulation and yield of the grass-legume mixture, as already reported by *Warembourg* et al. (1997). Nodulation and nitrogenaze activity is suppressed by high levels of available nitrogen in the soil, as proposed for forage legumes by *Mengel* et al. (2001) and by *Chmelíková* and *Hejcman* (2012b; 2013) for clover species. Generally, red clover responded less to nitrogen availability in soil in comparison to alfalfa. As far as can be concluded from our study design, plant age seemed to express more in above-ground traits of red clover than in its below-ground traits (*Chmelíková* et al., submitted).

Above-ground organs of red clover

The three cuts in 2011 revealed the seasonal development of above-ground organs of red clover in the grass-legume mixture. This development was associated with changes in stand structure and yield as well as nodule and root traits.

Plant height and the number of stems were affected by time within the season. At time of the second cut plant height was larger than at time of the first cut. This finding agrees with results of *Shen et al.* (2013) and was probably due to rapid stem growth from the stubble.

Above-ground plant traits (e.g. plant height and number of stems) contribute to yield production. Yield was positively related to plant height as described by *Užík* (1975). *Drobná* (2009) recorded the highest plants at sites with the highest yield production. The largest observed plant height (70.6 cm) was recorded for the second cut. By contrast, *Fan* et al. (2004) recorded the largest heights (70 cm) at time of the first cut. *Tucak* (2009) and *Asci* (2011) investigated the plant height of red clover cultivars (average height: 57.3 cm; range: 32.7-66.7 cm). Further, *Muntean* (2006) found that the height of the clover plants in the first year of growth was smaller than in the second year. These results seem to be inconsistent with the results at site D1, where the plants were smaller than the younger plants at the other sites. However, the effect could not be determined because of the interaction between plant age and site conditions. Results can nevertheless be affected by differences between a monoculture and a species mixture. In monocultures e.g. *den Hollander* et al. (2006) recorded higher plants than in a mixture. Additionally there may be an influence of the change in the proportion of legumes or the increase in grasses by the year of vegetation, as suggested by *Fin* et al. (2013). Comparing site D1 with the other sites we found a slight change in the composition of the mixture with time. Nevertheless, the highest and the shortest plants of red clover were growing on the same sites like the highest plants of alfalfa (*Chmelíková* et al., submitted).

Fan et al. (2004) observed the highest increase of dry matter accumulation during the stem branching and flower formation. Our results showed no clear positive relationship between the number of stems per plant and yield. There was no positive relationship between the number of inflorescences per plant and yield. On average there was a considerable decrease in biomass between the first (45% of total yield), second (36% of total yield) and third (19% of total yield) cut (*Chmelíková* et al., submitted). The regrowth of red clover decreased with repeated cutting at the same reproductive stage. *Fan* et al. (2004) described the outcome in plant height as a clear phenomenon due to the proportional reduction of regrowth days at the peak of the growing season.

Stem number per plant increased with age and with number of cuts. One of the sites with higher number of stems was D1, where plants were older. The highest numbers of stems per plant were however recorded at sites with one year old plants. Many authors (*Peterson* et al., 1992; *Taylor* and *Quesenberry*, 1996; *Sengul*, 2002) described the positive effect of age on stem number as well. According to *Singh* and *Winch* (1974) the number of stems increased markedly soon after cutting. With the exception of D1 and V2 the highest stem number was recorded at time of the final cut, what is in line with results by *Taylor* and *Quesenberry* (1996). The number of stems in red clover increased after cutting as the number of stems in alfalfa (*Chmelíková* et al., submitted). According to *Sheaffer* et al. (2000) this is connected with low leaf production. *Taylor* and *Quesenberry* (1996) additionally found the number of stems, leaves and petioles increasing with age as well. However, our results showed no relationship between higher number of stems and the lengths of the leaflet or the petiole. The lengths of leaf petiole and of the terminal leaflet increased with time. The same effect of seasonal leaf development was recorded for alfalfa as well (*Chmelíková* et al., submitted).

With exception of the site D1 the above-ground plant traits were clearly affected by locality and time. This is similar to results of *Queen* et al. (2009), who found the influence of location and year of vegetation on plants of red clover to be higher than the influence of light penetration through canopy and soil moisture. Because Site D1 differed in many factors like plant age, nutrient in the soil etc. it was not compared to the other sites.

The highest values of the most above-ground plant traits were observed on haplic cambisol suggesting a positive influence of the soil type. *Pardo* (2000) as well as *Kristofferesen* and *Riley* (2005) described the importance of the soil type for good persistence of red clover. The soil type influenced the nutrient and water uptake and was further reflected in the plant morphology.

The most inflorescences were recorded at the time of the second cut. Similar results were observed by *Elgersma* et al. (2000). The number of inflorescence was affected by time within the season. The majority of inflorescences was recorded in July at all sites. This is in accordance with the flowering time of red clover. With the exception of site D1 only a few inflorescences were recorded in May and August because the earliest month of flowering for the species is May (*Fitter* and *Peat*, 1994).

In conclusion we provide important insights about above- and below-ground traits of red clover and its ability of N_2 fixation. In light of future challenges to agriculture, like e.g. scarcity of resources and land, climate change and biodiversity loss, the utilization of ecosystem services will gain more attention. Results of this study are thus not only interesting from a scientific perspective, but also from a practical agronomic point of view. Organic as well as conventional farmers can use these findings in the future to increases benefits from legume crops and improve their overall biomass yields.

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Table 1: Description of study sites.

	D1	S1	S2	V1	V2	V3
Altitude (m a.s.l.)	450	470	470	480	480	480
Mean annual temperature (°C)	7.8	8.4	8.4	7.8	7.8	7.8
Mean annual precipitation (mm)	800	800	800	790	790	790
Soil type	Stagnic Fluvisol	Haplic Cambisol	Haplic Cambisol	Cambisol	Cambisol	Cambisol
Sand (%)	4	37	43	25	57	18
Silt (%)	71	45	41	58	31	51
Clay (%)	25	18	16	17	12	31
pH[1]	7.3	5.6	5.8	6.3	6.2	7.1
P (mg/100g)[2]	2.6	4.8	6.2	2.2	3.1	7.0
K (mg/100g)[2]	1.7	12.5	10.0	8.3	8.3	10.0
B (mg/kg)[3]	0.3	0.1	0.1	0.3	0.1	0.1
Cu (mg/kg)[3]	4.8	2.9	4.2	3.1	2.2	2.6
Fe (mg/kg)[3]	26	144	181	113	113	94
Mn (mg/kg)[3]	4.7	296	191	314	213	232
Zn (mg/kg)[3]	6.7	9.3	19.4	3.4	3.3	3.9
Mo (mg/kg)[4]	0.11	0.03	0.03	0.03	0.04	0.05
C organic (g/kg)[5]	111.7	9.6	8.8	11.4	11.2	10.2
N total (g/kg)	8.3	1.0	0.8	1.2	1.2	0.9
C : N ratio	9.45	9.6	10.90	9.62	9.64	11.50
N uptake by grass (g)[6]	1.81	0.99	0.84	1.05	1.07	1.03
Age (in years)	2	1	1	1	1	1
Proportion of legumes on herbage yield (%)	60-70	50-70	40-70	60-85	60-75	60-75
DM of total herbage yield (t ha^{-1})	8.0	11.0	10.2	11.9	8.8	11.7
DM of below-ground organs (t ha^{-1})	2.7	2.2	1.6	2.3	2.3	2.1

All measurements were according to VDLUFA (1991) standards: [1]measured in a suspension with 0.01 M $CaCl_2$; [2]extracted by a calcium-acetate-lactate solution (CAL); [3]measured by the CAT extraction method; [4]determined by the hot water extraction (HWE) method; [5]determined by the Dumas dry-combustion method; [6]measured in pot experiment – estimation of N availability in the soil via N uptake by *Lolium perenne* from 10 kg of the soil, extracted for nitrate (0.01 M $CaCl_2$, 1:5 soil-to-extraction ratio), analyzed photometrically after separation by HPLC.

Table 2: Summary of below-ground traits of red clover plants. One-way ANOVA was applied to evaluate the significance of the site effect. P – probability value obtained by the F test. Differences between sites denoted by the same letter (a–d) or differences between cuts denoted by the same letter (A–C) were not significantly different at the 0.05 probability value, calculated by post-hoc comparison using the Tukey HSD test.

Trait	Date	D1	S1	S2	V1	V2	V3	Mean	P
Diameter of the root neck - Diamet (mm)	May	8.7	5.5^{aA}	6.7^{bA}	5.6^{abA}	5.6^{abB}	5.8^{abA}	5.8	**0.032**
	July	11.1	7.0^{abB}	8.7^{bAB}	6.0^{aA}	7.5^{abA}	7.7^{abB}	7.4	**0.003**
	August	7.8	9.8^{cC}	9.1^{bcB}	7.9^{abB}	8.0^{abcA}	6.9^{aAB}	8.3	**0.001**
	MEAN	9.2	7.4	8.1	6.5	7.0	6.8		
	P		**<0.001**	**0.017**	**<0.001**	**<0.001**	**0.004**		
Maximal order of branching - OrdBran	May	3.4	3.5^{aA}	4.3^{bA}	4.2^{abA}	3.6^{abA}	3.9^{abA}	3.9	**0.005**
	July	3.8	2.3^{bB}	4.3^{aA}	3.7^{aA}	3.8^{aAB}	3.7^{aA}	3.5	**<0.001**
	August	4.3	3.3^{aA}	3.4^{aB}	3.1^{aB}	4.2^{bB}	3.4^{aA}	3.5	**<0.001**
	MEAN	3.8	3.0	4.0	3.6	3.9	3.6		
	P		**<0.001**	**0.001**	**<0.001**	**0.012**	0.082		
Number of nodules per plant - NodNr	May	12.5^{bA}	19.5^{aA}	19.5^{aA}	18.0^{aA}	19.5^{aA}	18.5^{aB}	18.0	**<0.001**
	July	14.0^{aA}	14.5^{abB}	17.0^{abcA}	17.5^{bcA}	18.0^{cA}	14.5^{abA}	16.0	0.212
	August	14.0^{abA}	17.5^{cdA}	19.5^{dA}	12.5^{aB}	16.0^{bcB}	13.5^{aA}	15.5	**<0.001**
	MEAN	13.5	17.0	17.5	16.5	18.0	15.5		
	P	0.146	**<0.001**	0.049	**<0.001**	**<0.001**	**<0.001**		
Size of nodules - NodSize	May	1.1^{aA}	1.3^{aA}	1.2^{aA}	1.5^{abA}	1.3^{aA}	1.8^{bA}	1.4	**<0.001**
	July	1.3^{aA}	1.2^{aA}	1.8^{aAB}	1.8^{aA}	1.5^{aA}	2.7^{bB}	1.8	**<0.001**
	August	1.4^{aA}	2.1^{aB}	2.0^{aB}	1.4^{aA}	1.6^{aA}	1.7^{aAB}	1.7	**0.027**
	MEAN	12.5	15.0	16.5	15.0	14.5	21.0		
	P	0.083	**<0.001**	**0.031**	0.346	0.482	**0.027**		
Cylindrical nodules - CylNod (%)	May	100^{aA}	100^{a}	100^{aA}	100^{a}	100^{a}	95^{aA}	99	0.421
	July	85^{aA}	100^{a}	90^{aA}	100^{a}	100^{a}	100^{aA}	96	0.048
	August	100^{A}	100	100^{A}	100	100	100^{A}	100	n.a.
	MEAN	95	100	97	100	100	98		
	P	0.042	n.a.	0.130	n.a.	n.a.	0.374		
Branched nodules - FanNod (%)	May	0^{aA}	0^{aA}	0^{aA}	0^{aA}	25^{bB}	10^{abA}	6	**0.001**
	July	0^{aA}	0^{aA}	0^{aA}	25^{abA}	0^{aA}	10^{abA}	3	0.212
	August	5^{aA}	10^{aA}	5^{aA}	10^{aB}	0^{aA}	0^{aA}	3	0.405
	MEAN	2	3	2	12	8	7		
	P	0.374	0.130	0.374	0.046	**0.003**	0.355		
Pink nodules - PinNod (%)	May	95^{aA}	100^{aC}	100^{aC}	100^{aA}	100^{aA}	100^{aA}	99	0.421
	July	85^{abcA}	65^{abB}	60^{aB}	95^{bcA}	75^{abcA}	100^{cA}	80	**0.005**
	August	80^{cA}	5^{aA}	5^{aA}	55^{bcB}	35^{abB}	40^{abB}	37	**<0.001**
	MEAN	90	60	60	83	70	80		
	P	0.377	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**		
Green nodules - GreeNod (%)	May	90^{aA}	100^{aA}	100^{aA}	95^{aA}	90^{aA}	100^{aA}	96	0.309
	July	70^{bB}	80^{abA}	75^{bA}	100^{aA}	100^{aA}	100^{aA}	88	**<0.001**
	August	85^{aA}	90^{aA}	80^{aA}	80^{aA}	85^{aA}	80^{aB}	83	0.949
	MEAN	81	90	90	92	90	90		
	P	**<0.001**	0.115	0.065	0.059	0.226	**0.012**		
Brown nodules - BroNod (%)	May	55^{bB}	10^{aA}	5^{aB}	0^{aB}	10^{aB}	0^{aA}	13	**<0.001**
	July	5^{cA}	50^{bB}	85^{aA}	40^{bA}	95^{aA}	100^{aC}	63	**<0.001**
	August	20^{bA}	100^{aC}	95^{aA}	70^{aA}	90^{aA}	80^{aB}	76	**<0.001**
	MEAN	30	50	60	37	70	60		
	P	**0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**		

Table 3: Summary of above-ground traits of red clover plants. One-way ANOVA was applied to evaluate significance of the site effect. P – probability value obtained by the F test. Differences between sites denoted by the same letter (a–c) and differences between cuts denoted by the same letter (A–C) were not significantly different at the 0.05 probability value calculated by *post-hoc* comparison using the Tukey HSD test.

Trait	Date	D1	S1	S2	V1	V2	V3	Mean	p
Height of the plant - PlHei(cm)	May	37.0	38.5^{bB}	35.1^{abB}	39.5^{bA}	29.0^{aA}	31.5^{aA}	34.7	**<0.001**
	July	38.1	50.8^{bA}	51.5^{bA}	70.6^{aB}	64.2^{aB}	68.6^{aB}	61.1	**<0.001**
	August	40.2	50.5^{cA}	55.9^{cA}	35.9^{bA}	29.2^{aA}	32.3^{abA}	40.8	**<0.001**
	MEAN	38.1	46.8	47.5	48.6	40.8	44.1		
	P		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**		
Number of stems per plant - NrStem	May	4.9	3.2^{aA}	3.5^{aA}	3.7^{aA}	3.5^{aAB}	3.3^{aA}	3.4	0.660
	July	4.2	4.3^{bA}	4.5^{bAB}	2.7^{aB}	2.9^{aA}	3.7^{abA}	3.6	**<0.001**
	August	4.1	6.3^{cB}	5.5^{bcB}	3.3^{aAB}	4.1^{aB}	4.1^{abA}	4.6	**<0.001**
	MEAN	4.4	4.5	4.5	3.2	3.5	3.7		
	P		**<0.001**	**0.003**	**0.010**	**<0.001**	0.080		
Length of the terminal leaflet - LeLe (cm)	May	2.7	3.5^{abB}	3.8^{aB}	3.7^{aA}	3.0^{bB}	3.7^{aA}	3.5	**0.004**
	July	3.3	4.6^{bA}	4.7^{bA}	3.9^{aAB}	3.8^{aA}	4.2^{abA}	4.2	**0.001**
	August	4.5	4.7^{aA}	4.8^{aA}	4.4^{aB}	3.7^{aA}	4.7^{aA}	4.4	0.311
	MEAN	3.5	4.3	4.4	3.9	3.5	4.2		
	P		**<0.001**	**<0.001**	**0.007**	**0.001**	0.413		
Length of the leaf petiole - LeafPet (cm)	May	16.7	25.2^{bB}	17.5^{aB}	24.4^{bA}	19.1^{aA}	21.6^{abA}	21.5	**<0.001**
	July	18.0	31.9^{bA}	29.9^{bA}	19.1^{aB}	18.9^{aA}	18.6^{aA}	23.7	**<0.001**
	August	22.7	30.8^{bA}	30.5^{bA}	23.4^{aA}	22.3^{aB}	21.7^{aA}	25.7	**<0.001**
	MEAN	19.1	29.3	25.9	22.3	20.1	20.6		
	P		**0.002**	**<0.001**	**0.007**	**0.002**	0.050		
Number of inflorescences per plant - Inflor	May	1.0	0.1^{aA}	0.2^{aA}	0^{aA}	0^{aA}	0^{aA}	0.0	0.497
	July	2.7	1.8^{aB}	1.4^{aB}	1.2^{aB}	1.4^{aB}	1.2^{aB}	1.4	0.741
	August	1.8	0.4^{bA}	0.4^{bA}	0.1^{aA}	0^{aA}	0^{aA}	0.2	**0.007**
	MEAN	1.9	0.8	0.7	0.4	0.5	0.4		
	P		**0.002**	**0.003**	**<0.001**	**<0.001**	**<0.001**		

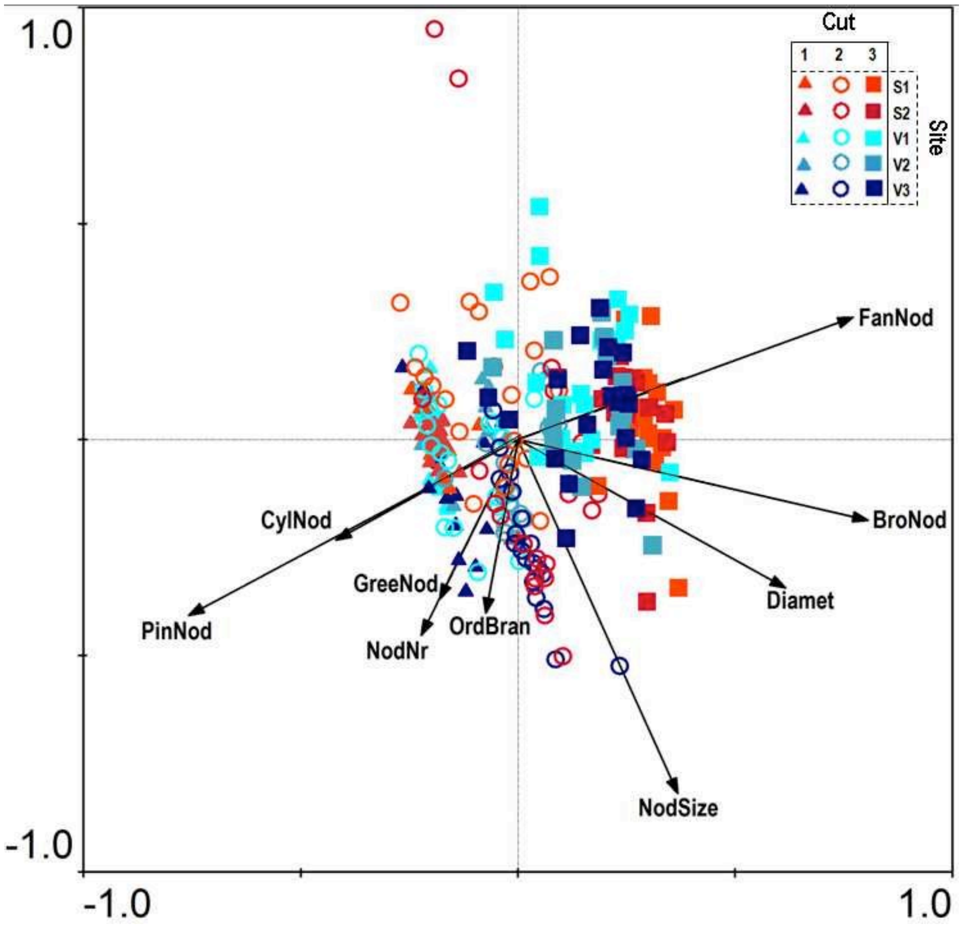


Figure 1: Ordination diagram showing the results of PCA of roots and nodule traits of *Trifolium pratense* at six sites (S1, S2, V1, V2, and V3– see Table 1 for site description). Plant trait abbreviations: BroNod – brown nodules; CylNod – cylindrical nodules; Diamet – diameter of the root neck; FanNod – fan-like nodules; GreeNod – green nodules; NodNr – number of nodules per plant; NodSize – size of nodules; OrdBran – maximum order of branching; PinNod - pink nodules.

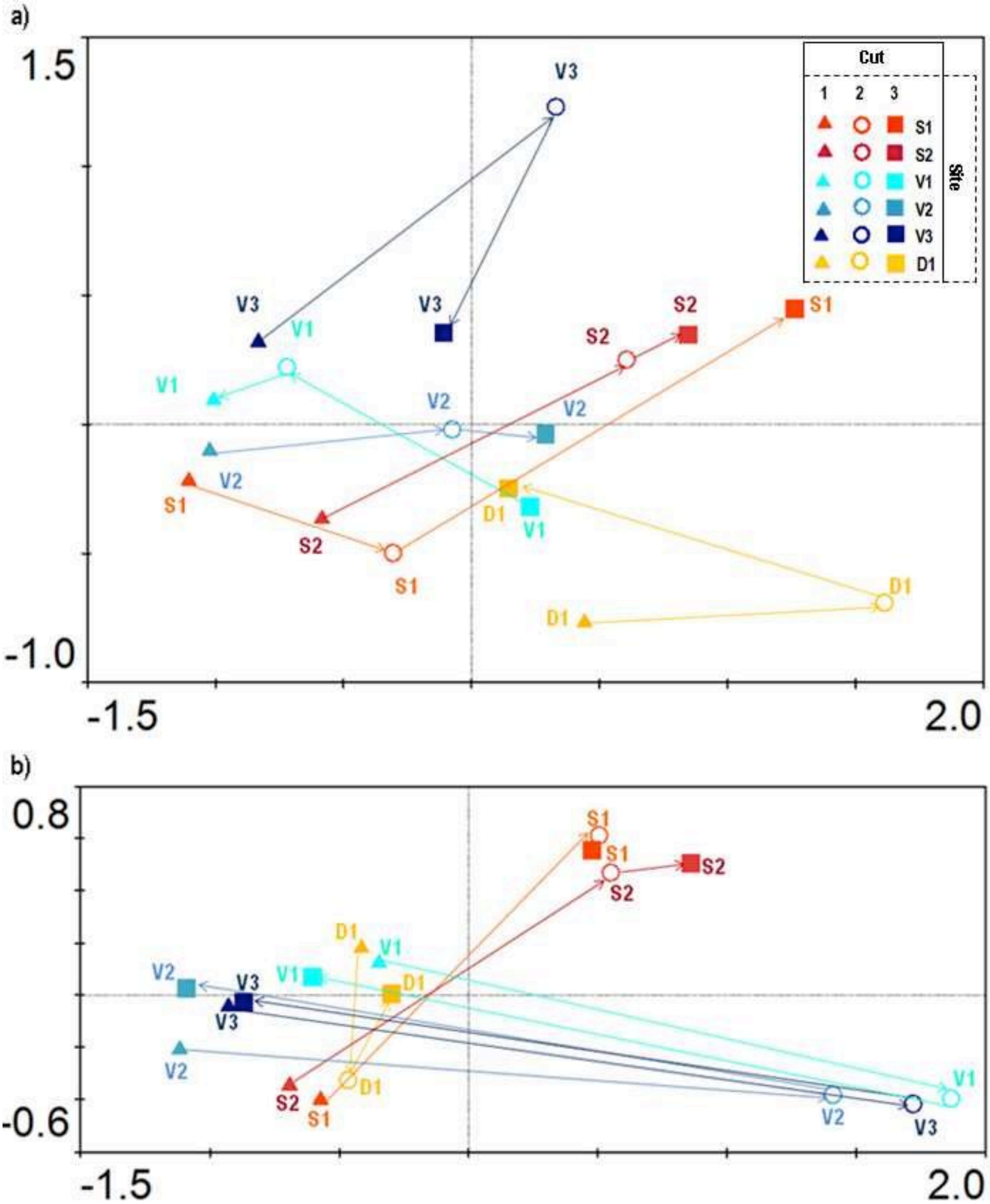


Figure 2: Principal component analysis (PCA) ordination (a) of below-ground organs and (b) of above-ground organs of *Trifolium pratense* on sites D1, S1, S2, V1, V2, and V3 in May (1), July (2) and August (3) 2011.

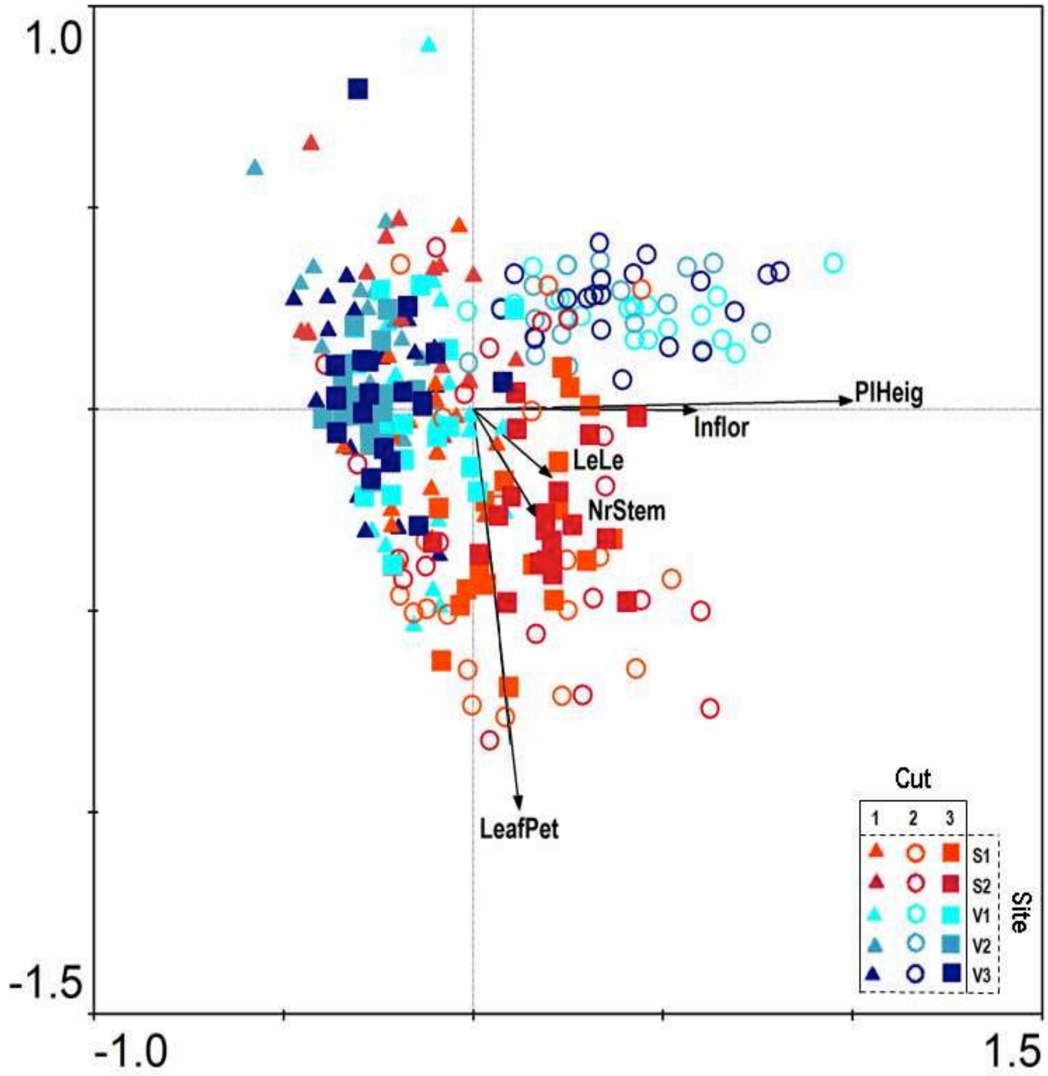


Figure 3: Ordination diagram showing the results of PCA of above-ground plant traits of *Trifolium pratense* at six sites (S1, S2, V1, V2, and V3- see Table 1 for site description). Plant trait abbreviations: Inflor - number of inflorescence; LeLe - length of the terminal leaflet; LeafPet - length of the leaf petiole; NrStem - number of stems per plant; PlHeig - height of the plant.

4. KOMENTÁŘE K VÝSLEDKŮM

V rámci disertační práce byly zkoumány kořenové systémy rostlin čeledi *Fabaceae* nejen na krajinné úrovni, ale také v rámci nádobového pokusu a na ekologicky obhospodařovaných zemědělských plochách. Při sběru dat byly užity následující metody: metoda monolitu, metoda půdní sondy, odkrývací metoda a metoda profilové stěny (viz. Příloha - Obr. 17 - 24). Výběr metody byl určen objektem výzkumu. Jednotlivé metody je často vhodné kombinovat, jelikož se výborně vzájemně doplňují. Popisy metod a jejich vylepšení na základě zkušeností v terénu jsou obsaženy v tezích disertační práce.

Na základě této obsáhlé a metodicky pestré disertační práce bylo možné dojít k celé řadě zajímavých poznatků:

I. Variabilita kořenových systémů běžných druhů leguminóz ve střední Evropě

U výzkumu čeledi *Fabaceae* na krajinné úrovni (214 rostlin, 21 druhů, 27 stanovišť) bylo zjištěno:

a) Kořenové systémy čeledi *Fabaceae* jsou velmi variabilní. Jejich variabilita byla dána především životní formou (RAUNKIAER 1934) a méně pak tribovou příslušností.

b) Kořenové systémy dosahovaly v kořenovém krčku průměru 0,1 - 1,2 cm. Nejnižší hodnoty byly naměřeny u jednoletých rostlin (*T. arvense*, *T. campestre*, *Vicia angustifolia*, *V. hirsuta*) a nejvyšší hodnoty u vytrvalých rostlin.

c) Šest druhů utvářelo kořenové systémy s rhizomy (*Genista tinctoria*, *Lathyrus pratensis*, *L. sylvestris*, *Securigera varia*, *T. medium*, *Vicia cracca*).

d) U 15 druhů dosahoval maximální řád větvení 3. řádu, u pěti druhů 5. řádu.

e) Kořenové systémy byly zabarveny bíle až tmavě hnědě. Jejich barva souvisela s životní strategií a stářím rostliny. Dřevnatění kořenů bylo pozorováno u 11 druhů rostlin.

f) Obnovovací pupeny byly pozorovány u 8 druhů.

g) Leguminózy utvářely hlízky kulatého, válcovitého, větveného, vějířovitě (prstovitě) větveného a nepravidelného (nádorovitého) tvaru.

h) Pouze jeden tvar hlízek byl nalezen u 11 druhů a dva tvary hlízek u 7 druhů rostlin. Tři až čtyři tvary hlízek byly pozorovány u tří druhů (*Astragalus glycyphyllos*, *G. tinctoria*, *V. hirsuta*).

i) Velikost hlízek se u devíti druhů pohybovala do 0,2 cm a u tří druhů dosahovala 0,2 - 0,4 cm. Hlízky větší než 0,4 cm byly nalezeny u devíti druhů.

Práce zabývající se 21 druhy čeledi *Fabaceae* na stanovištích s odlišnými podmínkami prostředí je jedinečnou studií, která zachycuje variabilitu kořenových systémů na krajinné úrovni. Tento výzkum však neumožnil určení přesné míry vlivu druhové příslušnosti a vlivu prostředí, jelikož se tyto vlivy navzájem překrývaly.

Variabilita kořenových systémů byla zaznamenána především z hlediska druhové příslušnosti. Mezidruhovou variabilitu bylo možné zkoumat na stanovištích, kde se nacházelo více druhů současně. Variabilita kořenových systémů v rámci druhu byla výrazně nižší než mezidruhová variabilita, což je v souladu s výsledky GALLOWAY ET FENSTER (2000).

K podmínkám prostředí, které se výrazněji projevily ve variabilitě kořenových systémů, patřila vlhkost půdy, dostupnost světla a polní podmínky. Na vliv právě těchto faktorů poukazují i jiní autoři (WAHL ET AL. 2001, SALAKO ET AL. 2002, ŠMILAUEROVÁ ET ŠMILAUER 2002, KOUKOURA ET AL. 2009). Na tomto místě je nutné poznamenat, že druhy vyskytující se na suchých stanovištích se liší od druhů rostoucích na vlhkých stanovištích. Rozdílné stanovištní nároky druhů tak neumožnily porovnat jednotlivé druhy podrobněji a určit míru jednotlivých vlivů na jejich variabilitu. Ačkoliv nelze oddělit vliv prostředí a druhu, je zcela evidentní, že vnitrodruhová variabilita byla podstatně menší než variabilita všech druhů. Nicméně i kořenové systémy rostlin náležících do stejného druhu rostoucí na stejném stanovišti se mohou lišit na základě genetiky. K přesnému porovnání vnitrodruhové a mezidruhové variability všech studovaných druhů by museli růst všechny druhy na všech studovaných lokalitách, čehož by bylo možné dosáhnout pouze přesazováním rostlin.

Životní forma dle RAUNKIERA (1934) hrála větší roli ve variabilitě kořenových systému než tribová příslušnost druhu, což bylo možné zjistit díky rostlinám různých životních forem náležících do jednoho tribu. Příkladem toho jsou jednoleté a víceleté rostliny tribu *Trifolieae* a *Viciaea*. Rostliny náležící tribu *Loteae*, *Galegeae* a *Genisteae* patřily pouze mezi pereny a jejich variabilita kořenových systémů byla tudíž relativně nízká. Rozdíly mezi kořenovými systémy jednoletých a víceletých druhů byly popsány ve více studiích (FITTER ET AL. 1988, GROSS ET AL. 1992, ROUMET ET AL. 2008). Dle našeho vyhodnocení kořeny jednoletých rostlin (*T. arvense*, *T. campestre*, *V. angustifolia* a *V. hirsuta*) tvoří přechod mezi kořenovými systémy s rhizomy a kořeny víceletých rostlin.

Vnitrodruhová variabilita kořenových systémů (schopnost plasticity rostlin) zvyšuje pravděpodobnost přežití jednotlivých rostlin v různých podmínkách prostředí. Jednotlivé znaky kořenových systémů se liší svoji plastičností v různých podmínkách prostředí, např. větvení kořenů je více plastické než průměr kořenového krčku. K podobným poznatkům došli také FORDE ET LORENZO (2001), kteří označili průměr kořenového krčku jako druhově specifickou vlastnost danou především geneticky, zatímco větvení kořene je závislé spíše na půdních podmínkách jednotlivých rostlin. Na základě výzkumu kořenových systémů na krajinné úrovni lze usuzovat, že některé znaky kořenových systémů jsou ovlivněny více podmínkami prostředí a jiné jsou dány spíše geneticky. K tomuto závěru došli i JOHNSON ET AL. (1996).

Životní forma se projevila v barvě kořenového systému. Většina kořenů byla zbarvena do světle hnědé barvy. Tato barva byla pozorovaná jak u jednoletých rostlin, tak u mladých kořenů víceletých rostlin. CORBY (1971) uvádí, že nejčastější barvou kořenů je právě světle hnědá barva. REJILI ET AL. (2007) pozoroval také vliv hlízek na barvu kořene, kdy obsah leghemoglobinu v hlízce zabarvil kořen, což v naší studii nebylo zaznamenáno.

Jako nejčastější tvar hlízek byl pozorován válec. V rámci naší studie byly posuzovány všechny hlízky - i malé hlízky kulovitého tvaru, které CORBY (1971) považoval za juvenilní a do výsledků je nezařazoval. V této práci byly hodnoceny hlízky všech tvarů a velikostí, aby nedošlo k chybnému označení malých kulovitých hlízek jako juvenilní. Kulovitý tvar hlízek byl například nalezen u *L. corniculatus* a *A. vulneraria*. Malé válcovité hlízky byly charakteristické pro rod *Trifolium* spp. Podle práce CORBY (1988) je pro jednotlivé triby charakteristický pouze jeden tvar hlízek. Dle našich výsledků bylo možné u některých tribů nalézt i více tvarů, ale pouze jeden z tvarů byly zastoupen nejčastěji. Ačkoliv bylo u tribů *Galegeae*, *Genisteae* a *Viciaea* popsáno více tvarů hlízek, vždy převažoval pouze jeden tvar nad ostatními. Většina hlízek se nacházela na tenkých (snadno infikovatelných) postranních kořenech, podobně jak popisuje CAPOEN ET AL. (2010). Tvary hlízek byly druhově specifické, jak zaznamenal REJILI ET AL. (2007). Výjimkou byly dvě rostliny druhu *T. arvense*, u kterých byly nalezeny větvené hlízky velmi odlišné od ostatních. Tyto rostliny se nacházeli na stanovišti spolu s *S. varia*, u které se běžně vyskytují větvené hlízky. Je tedy možné, že bakterie běžně infikující *S. varia* infikovala také kořeny *T. arvense*. Tyto bakterie by však měly být schopné díky genům rozpoznat vhodnost jednotlivých druhů rostlin.

Rozdíly v morfologii kořenových systémů souvisí s rostlinnou taxonomií. Podle ALLEN ET ALLEN (1981) náleží *S. varia* do tribu *Loteae*. Podle našich výsledků kořenových systémů a hlízek by *S. varia* měla být přiřazena spíše k tribu *Coronilleae*, podobně jako uvádějí ALLAN ET PORTER (2000).

Osm studovaných víceletých druhů rostlin (*A. vulneraria*, *A. glycyphyllos*, *C. scoparius*, *L. corniculatus*, *L. polyphyllus*, *M. lupulina*, *M. sativa* a *S. varia*) dosáhlo svým hlavním kořenem hloubky větší než 30 cm. Shodné údaje je možné nalézt i v rostlinných a kořenových databázích (FITTER ET PEAT 1994, KLIMEŠOVÁ ET DE BELLO 2009). Oproti víceletým rostlinám měli jednoleté rostliny (*T. arvense*, *T. campestre*, *V. angustifolia* a *V. hirsuta*), vytrvalé kořenové systémy s rhizomy (*G. tinctoria*, *L. pratensis*, *L. sylvestris*, *S. varia* a *V. cracca*) a některé víceleté rostliny s hlavním kořenem (*L. polyphyllus*, *M. albus*, *M. officinalis*, *T. medium* a *T. pratense*) většinou mělké kořeny nedosahující větší hloubky než 30 cm.

Velké množství jemných kořenů bylo pozorováno především u mělce kořenujících druhů. Tuto skutečnost lze vysvětlit jako adaptaci na suché podmínky prostředí, jak již popsal MERRILL ET AL. (2002).

Kořenové systémy běžně se vyskytujících druhů čeledi *Fabaceae* jsou velmi variabilní. Jejich variabilita je dána především životní formou dle Raunkiera a dále pak tribovou příslušnosti jednotlivých druhů.

II. Vliv dostupnosti dusíku, fosforu a draslíku na vzcházivost, nodulaci a růst acidofilního *Trifolium arvense* L. v alkalické půdě

Při výzkumu na základě nádobového pokusu bylo zjištěno:

a) Během vzcházivosti nebyly semenáčky *T. arvense* schopné získat dostatek fosforu.
b) *T. arvense* nekvetl v prvním roce v žádné variantě hnojení. V prvním roce utvářel pouze přízemní listové růžice. Ve druhém roce rostliny kvetly pouze ve variantách hnojených P, což naznačuje dvouletý charakter druhu. Semenáčky *T. arvense* nebyly schopné bez dodání P dalšího vývoje z juvenilního stádia.
c) Délka kořene dosahovala od 5 cm (varianta N2) do 12 cm (varianta P).
d) Průměr kořenového krčku a řád větvení byl průkazně ovlivněn variantou hnojení.
e) Nodulace byla pozitivně ovlivněna dostupností fosforu a negativně dostupností dusíku. Ve variantě N2 byla nalezena nejvýše jedna hlízka, ve variantách s P bylo zaznamenáno v průměru 13 hlízek.
f) Hlízky malého vzrůstu byly nalezeny v kontrole, ve variantě hnojené K a ve všech variantách s N. Největší velikosti dosahovaly hlízky ve variantě P1 a P2.
g) Válcovité, větvené a prstovitě větvené hlízky byly pozorovány ve variantách P1 a P2. Větvené hlízky byly nalezeny i ve variantách, kde byl fosfor v kombinaci s dusíkem a draslíkem.
h) Ve všech variantách byly nalezeny válcovité hlízky. Ve variantě N2 mělo hlízky jen 20 % rostlin, v kontrole pak 40 % rostlin.
i) Ačkoliv *T. arvense* roste na živinově chudých půdách, nebyla u něho ve variantách s živinami pozorována toxicita.

V rámci nádobových pokusů byl zkoumán vliv dostupnosti živin na *T. arvense* a *T. medium*. Vliv devíti variant hnojení (+ kontrola) byl posuzován jak u nadzemních, tak u podzemních částí rostlin. Nadzemní a podzemní části rostlin jsou v určitém vztahu, např. STURZ ET AL. (1997) popisuje korelaci mezi růstem nadzemní částí rostliny a nodulací u *T. pratense*. MONTPETIT ET COULMAN (1991) popsali vztah mezi adventivními kořeny a kvetením.

Spolu se stavbou rostlin byla hodnocena i vzcházivost obou druhů.

Vliv dostupnosti živin se projevil nejen ve vzcházivosti a pozorovaných znacích nadzemních částí rostlin *T. arvense*, ale také ve stavbě kořenového systému a v nodulaci. Při zvýšené dostupnosti fosforu utvářeli rostliny relativně rozsáhlé kořenové systémy v porovnání s ostatními variantami dostupnosti živin. Tento pozitivní vliv fosforu na velikost kořenového systému byl zaznamenán nejen u leguminóz, ale i u jiných druhů rostlin (LYNCH ET BROWN 2001, ADAMS ET AL. 2002, DUNBABIN ET AL. 2004, RAGHOTHAMA ET KARTHIKEYAN 2005, HILL ET AL. 2006, SVOBODA ET HABERLE 2006, PANG ET AL. 2010).

Nejvýraznější pozitivní vliv fosforu byl pozorován u nodulace, zároveň byl také zaznamenán i nepřekvapivý negativní vliv dostupnosti dusíku, což je v souladu s výsledky pracemi BRAUER ET AL. (2002), ROUMET ET AL. (2008) a LI ET AL. (2011). Fixace vzdušného dusíku je pro rostlinu energeticky náročný proces. Pokud má rostlina možnost přijmout jiné dostupné formy dusíku, tak sníží nodulaci nebo ji zcela inhibuje.

Neschopnost *T. arvense* přijmout dostatek fosforu v alkalické půdě se projevila negativně při fixaci vzdušného dusíku. Ke stejnému závěru dospěli také HØGH-JENSEN ET AL. (2002) pro *T. repens*, kdy při suboptimálním příjmu fosforu, došlo k poklesu fixace dusíku. Zvýšená dostupnost fosforu se naopak projevila zvýšenou nodulací.

Užité fosforečné hnojivo (superfosfát) obsahovalo síru, která podle VARIN ET AL. (2010) také podporuje nodulaci. Dostupnost síry se však mohla projevit i při vzcházivosti a při vývoji nadzemních a podzemních orgánů rostlin.

Více tvarů hlízek na jedné rostlině u *T. arvense* bylo vzácně zaznamenáno na stanovištích s nízkou dostupností fosforu (KUTSCHERA 1960, CHMELÍKOVÁ ET HEJCMAN 2012). Na krajinné úrovni dominovaly především hlízky válcovitého tvaru. Větvené hlízky se pravděpodobně mohou utvářet za vysoké dostupnosti fosforu.

Acidofilní chování *T. arvense* je pravděpodobně způsobeno neschopností semenáčků přijmout dostatek P z alkalické půdy. *T. arvense* v prvním roce nekvetl v žádné variantě. V druhém roce kvetl pouze ve variantách s P, což poukazuje na jeho dvouletý životní cyklus při pozdějším výsevu a jeho limitaci dostupností P na alkalických půdách. Bez dodání P rostliny *T. arvense* nebyly schopné se vyvinout ze stádia semenáčků. Nodulace byla pozitivně ovlivněna P a negativně N.

III. Vliv dostupnosti dusíku, fosforu a draslíku na vzcházivost, nodulaci a růst *Trifolium medium* L. v alkalické půdě

Při výzkumu *T. medium* na základě nádobového pokusu bylo zjištěno:

a) Vzcházivost u *T. medium* se pohybovala od 5 % v kontrolní variantě do 17 % ve variantě s P. Semenáčky vykazovaly zvýšenou citlivost na vysokou dostupnost N.
b) Nejvyšších hodnot dosahovaly znaky nadzemních částí rostlin ve variantách s aplikací všech živin (NPK) současně.
c) Byl pozorován negativní vliv dusíku na nodulaci, především ve variantách N1 a N2 bez aplikace fosforu. Počet hlízek na rostlinu byl nejnižší ve variantách s N a nejvyšší ve variantách s P.
d) Délka hlavního kořene byla v rozmezí od 11,5 cm (varianta N2) do 40,2 cm (varianta P1). Průměr kořenového krčku dosahoval 0,2 cm ve variantě N2 a 0,7 cm ve variantě hnojené P1. Maximální řád větvení byl nejnižší ve variantě N2 (2,3) a nejvyšší ve variantě P1 (3,9).
e) Největší hlízky byly nalezeny ve variantách s K a P, nejmenší v N2. Ve variantách N1 a N1P1 byly hlízky zaznamenány pouze u 14 % a 17 % rostlin.
f) *T. medium* je relativně vhodným druhem pro oblasti s alkalickou půdou. Pro dosažení maximálního vzrůstu vyžaduje tento druh, kromě zvýšené dostupnosti fosforu a draslíku, také zvýšenou dostupnost minerálního dusíku.

Vzcházivost *T. medium* byla stejně jako u *T. arvense* (viz. CHMELÍKOVÁ ET HEJCMAN 2012B) pozitivně ovlivněna dostupností P. Dostupnost fosforu se pozitivně projevila také v růstu a vývoji kořenových systémů *T. medium*. Nepatrně menší pozitivní vliv byl zaznamenán ve variantách s kombinací všech aplikovaných živin (NPK). Nejdelší hlavní kořeny u *T. medium* byly naměřeny ve variantách se zvýšenou dostupností fosforu, jak již popsal také ve své práci BALIGAR (1987). Ve variantách s dostupností fosforu a všech živin zároveň (NPK) došlo k nárůstu průměru kořenového krčku oproti ostatním variantám. Tento účinek byl pozorován jak u leguminóz (HILL ET AL. 2006, PANG ET AL. 2010), tak i u ostatních druhů rostlin (WAHL ET AL. 2001). Dle FAGERIA ET AL. (2014) podporuje dostupnost fosforu růst kořene a zároveň také příjem ostatních makro- (N, Ca, K, Mg, P) a mikroživin (Fe, Mn, Zn, Cu), což dále podporuje celkový růst a vývoj rostlin.

Vliv fosforu se také projevil ve větvení kořene. Ve variantách s P a K (varianty P1, P2 a K) se kořeny větvili více oproti jiným variantám. FORDE ET LORENZO (2001) popsali větší větvení kořenových systémů při současné aplikaci dusíku a fosforu. Kombinace těchto dvou prvků se však v rámci nádobového pokusu ve větvení neprojevila. Nejméně se kořeny větvily ve variantě N2. Aplikace dusíku měla vliv také na snížení růstu kořenového systému do hloubky, podobně jak popsali SVOBODA ET HABERLE (2006) u *Triticum aestivum*.

Negativní účinek N na nodulaci se projevil ve variantách (N1 a N2), kde byl aplikován bez P a K. Pravděpodobně zde došlo k limitaci nedostupností fosforu. Tato skutečnost byla pozorována také v kontrolní variantě. Dostupnost P, se podobně jako u *T. arvense,* projevila u *T. medium* velmi pozitivně při nodulaci. Tato reakce na dostupnost zmíněných živin byla zaznamenána mnoha autory (ADAMS ET AL. 2002, BRAUER ET AL. 2002, ROUMET ET AL. 2008, LI ET AL. 2011, CHMELÍKOVÁ ET HEJCMAN 2012B) i pro jiné druhy rostlin z čeledi *Fabaceae*.

Obecně platí, že při aplikaci dusíku závisí četnost a míra nodulace na dostupnosti fosforu. K podobným poznatkům došli u *Stylosanthes humulis* také GATES ET WILSON (1974). Současná aplikace dusíku a fosforu se projevila zvýšením nodulace, ale při aplikaci pouze dusíku byl vliv na nodulaci negativní.

Na základě pokusu s dostupností živin se *T. medium* zdá být vhodnou leguminózou pro pěstování na alkalických půdách. Při dobré dostupnosti P a K dosahuje *T. medium* i na alkalických půdách maximálního vzrůstu. Kromě P a K vyžaduje tento druh také určité množství N, čímž se liší od druhu *T. arvense* z předchozí studie (CHMELÍKOVÁ ET HEJCMAN 2012B). Vysoká dostupnost minerálního N zároveň s nízkou dostupností P a K zvyšuje mortalitu u semenáčků, snižuje růst nadzemních a podzemních částí rostlin a také nodulaci.

Při výzkumu dostupnosti živin se metoda nádobového pokusu zcela osvědčila. Především díky snadné manipulaci a zisku rostlinného materiálu. Je nutno podotknout, že při těchto pokusech hraje důležitou roli substrát a při venkovním nádobovém pokusu hrozí při vydatných deštích zatopení nádob.

IV. Sezónní vývoj biomasy jetelovinotravní směsi a vývoj nad- a podzemních orgánů *Medicago sativa* na rozdílných půdách

Při výzkumu *M. sativa* na zemědělských plochách bylo zjištěno:

a) Celkový výnos jetelovinotravní směsi se pohyboval mezi 8 – 12 t ha^{-1}, kdy podíl první, druhé a třetí seče byl 45, 36 a 19 %.

b) Podzemní biomasa jetelovinotravní směsi ve svrchních 30 cm půdy dosahovala 1,3 - 3,8 t ha^{-1}, u podzemní biomasy nebyl zaznamenán žádný průkazný vývoj v čase.

c) Průměr kořene a maximální řád větvení *M. sativa* se průkazně lišily v závislosti na stanovišti. Průměr kořene vzrůstal s časem.

d) Více větvené kořenové systémy se nacházely v půdách s vyšším obsahem písku.

e) Kořenové systémy s větším průměrem kořenového krčku utvářely menší hlízky.

f) Více větvené kořenové systémy utvářely více hlízek (nezávisle na jejich tvaru).

g) Počet hlízek klesal od května (9,5 - 17,0) do srpna (7,5 - 13).

h) Na začátku vegetační sezóny bylo zaznamenáno nejvíce aktivních hlízek na všech stanovištích.

Výzkum *M. sativa* a *T. pratense* (CHMELÍKOVÁ ET AL. SUBMITTED B) probíhal na plochách s jetelovinotravní směsí. Většina studií zabývající se *M. sativa* byla provedena v monokulturách a právě tím se naše práce odlišuje od ostatních a do jisté míry také komplikuje. U rostlin *M. sativa* a *T. pratense* byly hodnoceny jak nadzemní, tak podzemní části. U jetelovinotravní směsi byla hodnocena nadzemní a podzemní biomasa, aby sledované znaky mohly být začleněny do širšího kontextu práce a mohl být také hodnocen vztah mezi jednotlivými parametry. Např. WAREMBOURG ET AL. (1997) naměřil vyšší výnos na lokalitách s vyšším počtem hlízek u *T.pratense*.

M. sativa a *T. pratense* byly zkoumány současně na šesti plochách v ekologicky obhospodařovaných zemědělských podnicích. Tyto výzkumné plochy s téměř identickými klimatickými podmínkami byly shodně obhospodařované, lišili se pouze svými půdními podmínkami.

Na jedné ze šesti ploch (plocha D1) byly rostliny o rok starší než na ostatních plochách. U těchto rostlin nebylo možné oddělit vliv jejich stáří (užitkového roku) od vlivu stanoviště. Z tohoto důvodu nebyla plocha D1 porovnávána s ostatními. Vzhledem k náročnosti sběru dat a zajímavým zjištěním byla však data z plochy D1 v práci ponechána a pouze diskutována. Jak poukazují studie SUZUKI (1991), PIETOLA ET SMUCKER (1995) a HAKL ET AL. (2011), stáří rostliny se pravděpodobně projevilo například v průměru kořenů či jejich větvení. Tento vliv ale nebylo možné v naší práci přesně rozlišit a také nebyl cílem našeho výzkumu.

Nejnižší výnos nadzemní biomasy (8 t ha^{-1}) byl zaznamenán na ploše s nejvyšším obsahem organické hmoty v půdě a zároveň nejvyšší dostupností dusíku (plocha D1). BRAUN ET AL. (2010) naměřili na této ploše s identickou směsí v prvním užitkovém roce výnos 16 t ha^{-1}, což poukazuje na vysoký potenciál tohoto stanoviště a pokles výnosu mezi prvním a druhým rokem, jak zaznamenali také ALBAYRAK ET TÜRK (2013).

Nejvyšší výnosy byly naměřeny během první seče. Nejnižší výnosy byly dosaženy během třetí seče, což odpovídá běžným výnosům během vegetačního období ve střední Evropě (HREVUŠOVÁ ET AL. 2009, GIERUS ET AL. 2012, HAKL ET AL. 2012A). Rozdíly ve výnosech jednotlivých sečí (mezi jednotlivými roky) byly pravděpodobně způsobeny změnami v druhovém složení, kdy docházelo k dominanci některých druhů rostlin. Druhový poměr mezi travami a leguminózami v jetelovinotravní směsi se mění během vegetační sezóny (FIN ET AL. 2013, SANDERSON ET AL. 2013). Podíl jednotlivých druhů (např. *M. sativa*, *T. pratense*) na celkovém výnosu se tedy mezi sečemi liší (MARLEY ET AL. 2003). Ve výši výnosu se projevuje i fenologická fáze rostlin právě v době seče. Vliv fenologické fáze na výnos však nemusí být vždy průkazný, jak poukazují právě u *M. sativa* MARLEY ET AL. (2003).

Ačkoliv byly nalezeny rozdíly u druhé a třetí seči mezi jednotlivými stanovišti, tyto rozdíly se nijak nepromítli do podzemní biomasy. Podzemní biomasa se nelišila v místech s rozdílnými nadzemními výnosy. Dle našich výsledků investovali rostliny do podzemní biomasy nezávisle na množství nadzemní biomasy. Tyto výsledky jsou v rozporu s prací HAKL ET AL. (2011). LUO ET AL. (1995) zaznamenali vyšší podíl biomasy jemných kořenů na jaře a na podzim. Tyto výsledky částečně odpovídají vývoji během vegetační sezóny u čtyř ze šesti výzkumných ploch.

Biomasa jemných kořenů souvisí s maximálním řádem větvení. Nejvyšší řád větvení byl námi zaznamenán právě na jaře během první seče.

Rostliny na jednotlivých plochách se lišily řádem větvení a průměrem kořene. Tyto rozdíly poukazují na vliv půdních podmínek. Řád větvení a průměr kořene se měnily během vegetační sezóny. Vyšší řád větvení byl pozorován na lokalitách s vyšším obsahem písku v půdě, což bylo pravděpodobně spojeno se snazší penetrací kořene, podobně jak popisuje SALAKO ET AL. (2002).

Rostliny a jejich kořeny se vzájemně mezi sebou ovlivňují. HARPER (1977) upozorňuje na vliv hustoty a složení vegetace, kdy rostliny ovlivňují růst a morfologii sousedních rostlin, např. průměr kořene a počet laterálních kořenů, jak uvádí HAKL ET AL. (2011). Rozdíly v hustotě vegetace se při nízkém počtu sbíraných rostlin mohly projevit právě ve větší variabilitě znaků. Ačkoliv sběr rostlin probíhal na všech plochách jednotně je možné, že se nám nepodařilo tento vliv zcela eliminovat.

Díky krátkověkosti hlízek (PUPPO ET AL. 2005) nebyla nodulace pravděpodobně přímo ovlivněna stářím rostliny. Plocha D1 se staršími rostlinami byla tedy zařazena do hodnocení a porovnávání hlízek. Nepřímý vliv stáří rostlin lze však předpokládat. *M. sativa* je známá tím, že utváří silný hlavní kořen s menším množstvím laterálních kořenů (KUTSCHERA 1960, CHMELÍKOVÁ ET HEJCMAN 2012A). Starší rostliny utvářejí méně jemných kořenů, které jsou více náchylné k bakteriální infekci a následné nodulaci. Méně jemných kořenů na ploše D1 by mohlo být vysvětlením nižší nodulace u starších rostlin.

V mnoha studiích (LYNCH ET BROWN 2001, ADAMS ET AL. 2002, DUNDABIN ET AL. 2004, RAGOTHAMA ET KARTHIKEYAN 2005) je popisován vliv dostupnosti živin v půdě na nodulaci. Plochy s jetelovinotravní směsí se mezi sebou lišily dostupností živin (N, P, K, B, Cu, Fe, Mn, Zn, Mo), je tedy možné předpokládat určitý vliv živin na nodulaci. Za daných podmínek naší studie nebylo však možné rozpoznat vliv jednotlivých živin z důvodu jejich interakce a spolupůsobení. Na ploše D1 se staršími rostlinami byla v půdě naměřena vyšší dostupnost N. Vyšší dostupnost N způsobuje nižší počet hlízek a jejich menší velikost (ADAMS ET AL. 2002, BRAUER ET AL. 2002, BEEBE ET AL. 2006, GREWAL 2010, LI ET AL. 2011), přesto je nutno zohlednit i ostatní vlivy stanoviště (např. nižší obsah kyslíku v těžší půdě, stáří rostlin aj.) a komplexnost působení všech vlivů zároveň.

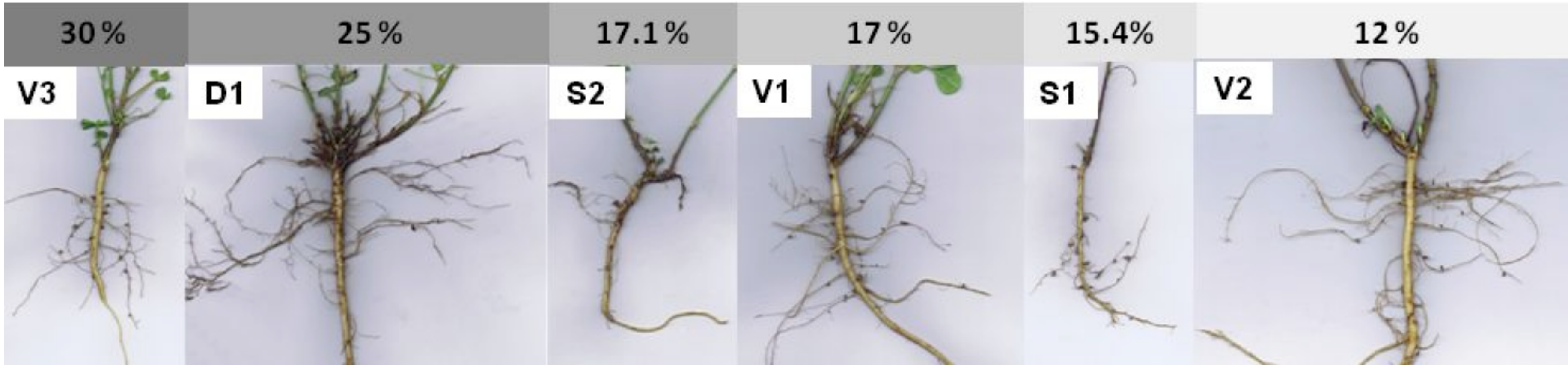
Na kořenech *M. sativa* je možné pozorovat hlízky válcovité a také větvené. Jak již bylo popsáno v práci CHMELÍKOVÁ ET HEJCMAN (2012A), větvené hlízky se utvářejí v podmínkách s lepší dostupností P a K a nižší dostupností N (CHMELÍKOVÁ ET HEJCMAN 2012B). Plocha s o rok staršími rostlinami se odlišovala od ostatních ploch svoji výrazně nižší dostupností draslíku a vyšší dostupností minerálního dusíku. Na této ploše se utvářelo jen malé množství drobných hlízek, především válcovitého tvaru. Snížená nodulace u starších rostlin s vyšší dostupností dusíku by mohla být vysvětlena také snížením podílu leguminóz na ploše a nárůstem druhů trav ve druhém roce.

Rozdílná dostupnost molybdenu v půdě se projevuje v počtu a velikosti hlízek u *M. sativa*, stejně jako u *G. max* (VIEIRA 1998, TOLEDO ET AL. 2010). Tento pozitivní vliv nebyl v naší studii zaznamenán. Důvodem toho byla pravděpodobně dostačující dostupnost molybdenu v půdě na všech lokalitách. V průběhu vegetační sezóny byl zaznamenán nejnižší počet stárnoucích a odumírajících hlízek na lokalitě s nejvyšším obsahem molybdenu v půdě. Delší doba trvání aktivní nodulace mohla být podpořena právě vyšším obsahem molybdenu na této ploše. K podobným výsledkům došli i SHAW ET AL. (1966).

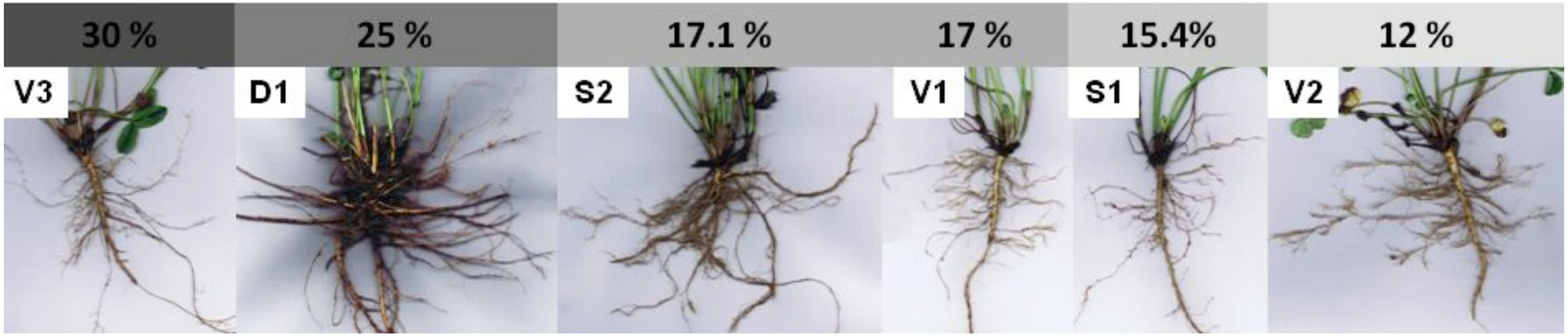
Pokles počtu hlízek během vegetace je v souladu se závěry práce ZAHRAN (1998). Počet hlízek klesal na všech lokalitách s výjimkou jedné. Důvodem opačného vývoje by mohl být vyšší obsah písku a dobrá dostupnost P, příp. opožděný začátek vegetační sezóny způsobený mrazem. Nejvyšší počet aktivních hlízek byl zaznamenán v květnu během první seče, většina neaktivních a odumírajících hlízek byla zaznamenána během třetí seče v srpnu. Stejný vývoj hlízek během vegetační sezóny zaznamenali také SWARAJ ET BISHNOI (1996).

Vliv dostupnosti živin se projevuje také změnami v morfologii kořenových systémů. U leguminóz se jedná především o fosfor, který mj. ovlivňuje průměr kořene, jak popsali PANG ET AL. (2010) u *M. sativa*. LYNCH ET BROWN (2001) a HILL ET AL. (2006) pozorovali v půdách s nižší dostupností fosforu více větvené kořeny u rodu *Glycine* spp. Opačný vliv pak popsali ADAMS ET AL. (2002), kdy se kořeny více větvily v půdě s vyšším obsahem fosforu. Rozdíly v dostupnosti fosforu i ostatních živin nebyly na našich výzkumných plochách výrazné, tudíž nebylo možné posoudit vliv jejich dostupnosti. Determinaci jednotlivých vlivů znemožňovalo také současné spolupůsobení více faktorů a jejich vzájemná interakce. Určení těchto vlivů na *M. sativa* nebylo objektem a tudíž ani cílem výzkumu.

Naskenované kořenové systémy druhů *M. sativa* a *T. pratense* jsou zachyceny na obrázcích 15 a 16.



Obr. 15: Naskenované kořeny *M. sativa,* červenec 2011 (2. termín). Procenta udávají podíl jílovité frakce v půdě na jednotlivých plochách.



Obr. 16: Naskenované kořeny *T. pratense*, červenec 2011 (2. termín). Procenta udávají podíl jílovité frakce v půdě na jednotlivých plochách.

V. Sezónní vývoj nad- a podzemních orgánů *Trifolium pratense* v jetelovinotravní směsi na rozdílných půdách

Při výzkumu *T. pratense* na zemědělských plochách bylo zjištěno:

a) Průměr kořenového krčku, řád větvení, velikost hlízek a počet stárnoucích hlízek byl ovlivněn půdními podmínkami během celé vegetační sezóny.

b) Průměr kořenového krčku vzrůstal během vegetační sezóny.

c) Kořenové systémy s menším průměrem kořenového krčku utvářely více aktivních hlízek.

d) Počet hlízek se během vegetační sezóny pohyboval v rozmezí 12,5 - 19,5 hlízek na rostlinu.

e) Válcovité hlízky byly nalezeny u 85 - 100 % rostlin na každé ploše.

f) U 0 - 25% rostlin byly nalezeny větvené hlízky.

g) Kořenové systémy *T. pratense* se větvily více než kořenové systémy *M. sativa*.

h) V porovnání s *M. sativa* utvářel *T. pratense* více hlízek na všech plochách.

i) Hlízky *T. pratense* byly menší než hlízky *M. sativa*.

Trifolium pratense byl zkoumán zcela identicky jako *M. sativa* (CHMELÍKOVÁ ET AL. SUBMITTED A). Podobně jako v předchozí studii nebylo možné ani u *T. pratense* oddělit vliv stáří rostlin od vlivu prostředí na ploše D1. O rok starší rostliny *T. pratense* se však od ostatních jednoletých rostlin neodlišovaly tak výrazně, jako tomu bylo u rostlin *M. sativa* (CHMELÍKOVÁ ET AL. SUBMITTED A).

Za stejných klimatických podmínek se pozorované znaky kořene (průměr kořenového krčku a řád větvení) *T. pratense* lišily v závislosti na výzkumné ploše, tedy podle půdních podmínek. Zároveň se znaky lišily i mezi jednotlivými sečemi. Při hodnocení nodulace byl zaznamenán větší vliv průběhu sezóny než vliv půdních podmínek.

Průměr kořenového krčku narůstal s časem stejně jako u *M. sativa*. Obecně se kořeny *T. pratense* více větví ve srovnání s *M. sativa* a nejinak tomu bylo i v naší studii. Ačkoliv se během vegetační sezóny řád větvení *T. pratense* mezi jednotlivými termíny lišil, nebyl zaznamenán žádný jednotný trend ve vývoji na všech stanovištích, jak tomu bylo u *M. sativa* (CHMELÍKOVÁ ET AL. SUBMITTED A).

Vliv půdní zrnitosti se projevil v hodnocených znacích kořene. Průměr kořenového krčku se lišil mezi jednotlivými plochami. Kořeny s větším průměrem byly zaznamenány na plochách s vyšším obsahem písku. Půdní zrnitost (podíl písku) souvisí s obsahem vzduchu v půdě. MELA (2003) pozoroval v dobře provzdušněných půdách u *T. pratense* silné kořeny a naopak kořeny s menším průměrem v jílovitých půdách.

Vyššího řádu větvení dosahovaly kořeny rostlin na plochách s vyšším podílem písku v půdě, na těchto plochách se více větvili i kořeny *M. sativa*. SALAKO ET AL. (2002) vysvětlují větvení kořenů v písčitých půdách možností snazší penetrace kořene. ARCHER ET AL. (2002) a GLAB (2008) zaznamenali naopak navýšení kořenové biomasy v kompaktnějších půdách. Kompaktnost půdy je ovlivněna orbou. Hloubka orby se projevila zúžením hlavního kořene u obou druhů - *M. sativa* i *T. pratense* (hodnoceno pouze vizuálně).

Homogenní rozložení kořenů je možné podle PARDO ET AL. (2000) v půdách s jemnou texturou. Textura půdy spolu s rozložením kořenů ovlivňuje schopnost rostlin přijímat vodu a živin, např. draslík. *T. pratense* je v porovnání s travami schopen přijmout draslík i z větších hloubek než trávy (SVEISTRUP ET HARALDSEN 1997, OGAARD ET HANSEN 2010). Podle JANSEN ET AL. (2010) vysvětluje půdní typ dokonce až 42% variability příjmu vody u *L. polyphyllus*. Reakce rostlin jsou však druhově specifické. FRENCH (2002) nezaznamenal vliv půdního typu na příjem vody u *Pisum sativum*.

Na kořenovém systému *T. pratense* se zřetelným hlavním kořenem byly pozorovány válcovité hlízky, jak uvádějí CORBY (1971), STURZ ET AL. (1997), KLIMEŠOVÁ ET DE BELLO (2009) a CHMELÍKOVÁ ET HEJCMAN (2012A). Nezávisle na stanovišti utvářel *T. pratense* více hlízek menší velikosti v porovnání s *M. sativa*. Stáří rostlin se pravděpodobně projevilo pouze nepřímo v nodulaci, podobně jako u *M. sativa*. VOISIN ET AL. (2002) a HATCH ET AL. (2007) popsali snižování fixace dusíku s přibývajícím věkem rostlin. Nižší nodulace byla skutečně nalezena na ploše se staršími rostlinami. Snížení nodulace však nelze přičíst pouze stáří rostliny. Pravděpodobně se zde projevil vyšší obsah minerálního dusíku v půdě, který snižuje, ba dokonce inhibuju nodulaci (BUTTERY ET AL. 1992, MENGEL ET AL. 2001, VOISIN ET AL. 2002, PEOPLES ET GRIFFITHS 2009, CHMELÍKOVÁ ET HEJCMAN 2012B, CHMELÍKOVÁ ET HEJCMAN 2013).

Nodulace je ovlivněna růstem a fenologickou fází rostliny. Oproti *M. sativa* nedošlo u *T. pratense* k poklesu počtu hlízek v průběhu vegetační sezóny, došlo však ke snížení jejich aktivity. Tyto výsledky jsou v souladu s pracemi JENSEN (1986), VOISIN ET AL. (2002) a CUPINA ET AL. (2010). BRAUER ET AL. (2002) zaznamenal zvýšenou tvorbu hlízek na půdách s vyššími hodnotami pH. Půdní reakce v rozmezí 5,6 - 7,3 se však ve změně nodulace neprojevila, jelikož *T. pratense* snáší dobře větší rozpětí hodnot pH.

Mnoho autorů (MARSCHNER 1995, LEITE ET AL. 2009, TOLEDO ET AL. 2010, FAROOQ 2012) poukazuje na důležitost molybdenu při fixaci dusíku a jeho pozitivní vliv na počet a velikost hlízek u leguminóz. GATES ET WILSON (1974) a XIA ET XIONG (1991) zaznamenali pozitivní vliv molybdenu v kombinaci s draslíkem na nodulaci *T. alexandrinum*. Na ploše s vyšší dostupností molybdenu byl sledován větší podíl aktivních hlízek v průběhu celé vegetační sezóny, shodně jako u *M. sativa*.

U *T. pratense* i *M. sativa* byly pozorovány jak průkazné vlivy průběhu vegetace, tak i vlivy půdních podmínek. Determinace jednotlivých vlivů nebyla možná, což však nijak nesnižuje význam výsledků pro jejich praktické užití.

5. ZÁVĚR

Předložená práce rozšiřuje a doplňuje poznatky o stavbě kořenových systémů a nodulaci čeledi *Fabaceae*. Díky své jedinečné schopnosti fixace vzdušného dusíku tvoří tato čeleď nenahraditelnou skupinu rostlin jak v přírodních ekosystémech, tak i na zemědělských plochách. V rámci disertační práce byly zkoumány kořenové systémy rostlin čeledi *Fabaceae* nejen na krajinné úrovni, ale také v rámci nádobových pokusů a na ekologicky obhospodařovaných zemědělských plochách. Spolu s kořenovými systémy byly hodnoceny i nadzemní části rostlin, zároveň byly zaznamenány podmínky prostředí, aby poznatky o kořenových systémech mohli být zasazeny do širších souvislostí a měly větší výpovědní hodnotu.

Kořenové systémy běžně se vyskytujících druhů čeledi *Fabaceae* na svých přirozených stanovištích poukázali na vnitrodruhovou a mezidruhovou variabilitu. Mezidruhová variabilita 21 druhů leguminóz byla připsána především životní formě dle Raunkiaera. Vnitrodruhová variabilita poskytla údaje o schopnosti adaptace jednotlivých rostlin a plastičnosti jejich znaků. Bližší pozornost byla dále věnována pouze 4 druhům.

Dostupnost živin byla hodnocena u *T. arvense* a *T. medium* v alkalické půdě. Acidofilní (kalkofobní) chování *T. arvense* nebylo dosud nikdy experimentálně zkoumáno. Stejně tak nebyl dostatečně prozkoumán vliv dostupnosti živin u *T. medium* v alkalických půdách. Acidofilní chování *T. arvense* bylo v rámci pokusu vysvětleno neschopností přijímat dostatek fosforu v alkalických půdách. Fosfor měl na růst a vývoj *T. arvense* zásadní vliv. Růst a vývoj *T. medium* byl také pozitivně ovlivněn dostupností P, ale největšího vzrůstu dosáhli rostliny ve variantách s aplikací všech živin (NPK) současně.

Jedny z celosvětově nejdůležitějších pícnin (*M. sativa* a *T. pratense*) byly zkoumány a porovnány v jetelovinotravní směsi na ekologicky obhospodařovaných zemědělských plochách s odlišnou zrnitostí půdy. Nadzemní biomasa se lišila v závislosti na půdních podmínkách a průběhu vegetační sezóny. Podzemní biomasa nevykazovala žádný z těchto vlivů. Více větvené kořenové systémy *T. pratense* utvářely více hlízek než méně větvené kořenové systémy *M. sativa*. Na začátku vegetační sezóny bylo u obou druhů zaznamenáno nejvíce aktivních hlízek na všech stanovištích. Odlišné půdní podmínky se projevily ve stavbě kořenových systémů.

Vzhledem k velkému množství faktorů ovlivňujících stavbu kořenových systému a k jejich variabilitě je vhodné i nadále ve výzkumu pokračovat.

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7. PŘÍLOHA

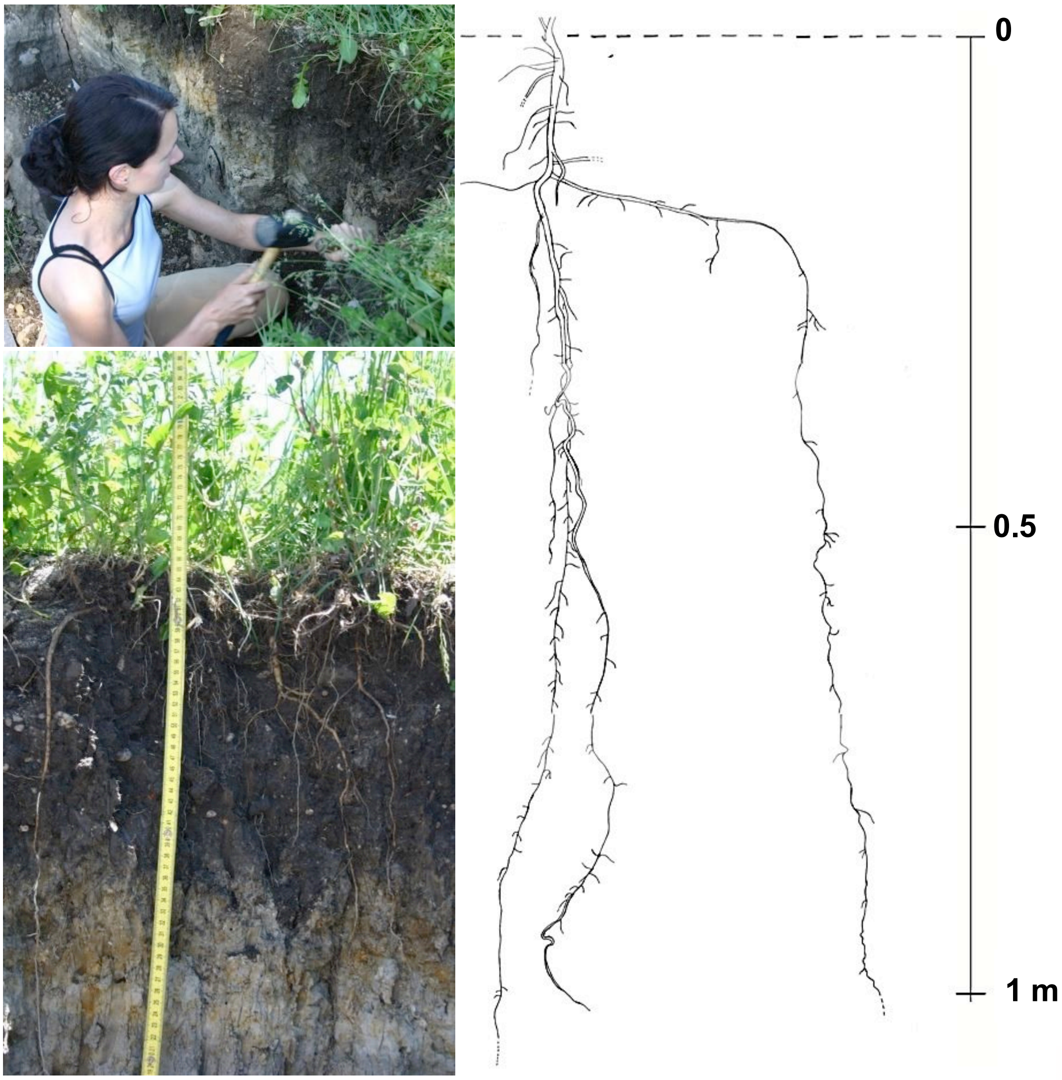
Obr. 17: Metoda monolitu - zarážení rámu do země.

Obr. 18: Metoda monolitu - rám s odebraným půdním monolitem.

Obr. 19: Metoda monolitu - manipulace a transport.

Obr. 20: Vymývání a preparace kořenových systémů.

Obr. 21: Metoda půdní sondy a kombinace metody půdní sondy s metodou profilové stěny.



Obr. 22: Odkrývací metoda - odkrývání, odkryté kořeny v půdním profilu a kořenový systém *M. sativa* (nákres získaný odkrývací metodou).

Obr. 23: Metoda profilové stěny - příprava výkopu a profilové stěny.

Obr. 24: Metoda profilové stěny - průběh metody profilové stěny a detail hodnocených kořenových délek.