Water potential gradient facilitated interplant transfer via *Desmanthus illinoensis*

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Dedication

This dissertation is dedicated to my late father and my late advisor Kenneth Norris, two men whose momentous contribution to science was only surpassed by their contribution to humanity.

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Chapter I: Literature Review

Mycorrhizal fungi

Mycorrhizal fungi are a ubiquitous component of soil biota found in nearly all terrestrial ecosystems. Mycorrhizal fungi form symbiotic associations with over seventy percent of all terrestrial plants species (Francis and Read, 1995). There is evidence the symbiosis dates back to the time when plants were making the transition from an aquatic to terrestrial environment over 400 million years ago (Remy et al, 1994; He et al, 2004). Mycorrhizal fungi are obligate biotrophs that utilize host plant derived photosynthates in exchange for soil-derived nutrients including phosphorous and nitrogen (Smith and Read, 1997). One of the most common types of mycorrhizal fungi is arbuscular mycorrhizal (AM). The majority AM fungal species lack host-specificity, allowing the AM fungi to form simultaneous symbiotic relationships with multiple plant species, which is believed to have allowed the fungi to colonize most terrestrial ecosystems (Smith and Read, 1997).

AM fungi colonize the host plant root cells forming unique structures such as arbuscules and vesicles. AM fungal hyphae do not penetrate the endodermis and therefore do not enter into the vascular system (He et al, 2003). Arbuscules are formed by highly branched hyphae within a plant cell which do not penetrate the plant protoplast. Arbuscules are believed to function as the primary site of nutrient exchange between symbionts (Smith and Read, (1997). AM fungi also form extraradical hyphae in the rhizosphere which help capture soil based nutrients such as nitrogen and phosphorus.

Based on overall biomass and photosynthate consumption estimates, mycorrhizal fungi are a major component of soil biota. There are projections that upwards of twenty percent of plant-derived photosynthates are consumed by AM fungi, on par with the amount the plant allocates to its own root system (Jacobson and Rosendahl, 1999). Correspondingly, the combined biomass of the intra- and extraradical mycelium has been estimated to equal the biomass of the host root systems (Olsson et al, 1999).

Ecological significance of mycorrhizal fungi

Mycorrhizal fungi are believed to provide an array of ecological services within ecosystems such as: assisting in seedling establishment (Borchers and Perry, 1990), assisting in restoration/reclamation following disturbance (Perry et al 1989), altering competitive dominance relationships within plant communities (Grime et al, 1987), reducing nutrient loss from ecosystems (Hamel et al, 1991), increasing productivity and sustainability of agroecosystems (Simard et al, 1997), accelerating decomposition rates (Hodge et al, 2001), and improving drought tolerance of the host plant (Simard, 2002).

Common Mycorrhizal Networks

AM fungi are capable of forming intra- and interspecific links between plant roots (Francis et al, 1986; Newman, 1988; Newman et al 1994). By definition, when mycorrhizal fungi link two or more plant roots a common mycorrhizal network (CMN) exists (Simard and Durall, 2004). The complexity of a CMN increases as the number of fungal species, linkages, and host species increase (Simard and Durall, 2004). The aseptate (lacking cross-walls) morphology of AM hyphae raises the potential that CMNs facilitate network resource sharing. Source/sink gradients are believed to be the primary factor influencing molecular movement within a CMN (Simard and Durall, 2004). CMNs have the potential influence plant survival and by affecting the nutrient status of plants sharing the network (Simard and Durall, 2004).

Interplant hyphal linkages in terrestrial grassland and forest ecosystems are presumed to be common even though it has not been conclusively proven because of challenges associated with visualizing intermingling 3-D hyphal linkages (Newman, 1988). The existence of mycorrhizal networks has been demonstrated in laboratory studies (Simard and Durall, 2004). The potential significance of mycorrhizal fungi in terrestrial landscapes underscores the need for understanding the factors that regulate network function as well as determining if AM fungi function as a direct conduit for interplant transfer.

Interplant transfer is enhanced in the presence of AM fungi, but is the pathway direct?

Ever since it was suspected mycorrhizal fungi form interplant root linkages there has been an effort to determine if molecular interplant transfer occurs. Several studies indicate enhanced molecular transfer occurs when plants share a CMN (Heap and Newman, 1980, Francis et al, 1986, Hamel and Smith, 1991; 1992, Azcon and Barea, 1993; Haystead et. al, 1998; Cheng and Baumgartner, 2004) yet the lack of a visible CMN combined with the lengthy exposure periods of the studies has made it difficult to discern if the enhanced transfer is occurring by an indirect or direct CMN route. An indirect interplant transfer pathway involves leaving the CMN at some point and entering the rhizosphere or ñsoil poolò.

Direct transfer pathway: a three step process

To best comprehend interplant transfer research and the challenges associated with it, a vital grasp of the complexities of a direct interplant CMN transfer route is required. For direct molecular transfer to occur between a donor and a receiver plant the following criteria must be met: (i) efflux, molecular transfer from the donor plant into the fungus; (ii), influx, molecular transfer from the fungal tissue into the receiver tissue; and (iii) translocation, molecular movement within the fungus, such as across a hyphal bridge. Arbuscules are believed to be the primary site of symbiotic transfer between the plant and the fungus (Dickson and Smith, 2001).

Arbuscules: a biotrophic interface and the primary site of molecular exchange

Arbuscules are believed to be the primary site of bidirectional molecular exchange between symbionts (Smith and Read, 1997). Plant-derived carbon passes from the autotroph to the heterotroph and soil derived nutrients such as phosphorus and nitrogen pass from the heterotroph to the autotroph across arbuscules. Arbuscules are created by dichotomous branching of fungal hyphae within plant cortical cells (Peterson and Massicotte, 2004), forming a biotrophic interface between the autotroph (plant) and the heterotroph (fungi). The biotrophic interface consists of a plant and fungal membrane separated by an interfacial apoplastic compartment. The interfacial apoplastic compartment is formed from the fungal cell wall which appears to be modified and plant-derived material (Smith and Read, 1997). The apoplastic compartment is not believed to inhibit molecular exchange between the symbionts.

Looking at other biotrophic interfaces for insight?

There are many unanswered questions vis-à-vis the molecular transfer mechanisms and pathways through the symbiotic interface. Elucidating mechanisms which can account for bidirectional molecular exchange between independent symbiotic membranes separated by an apoplastic space has confounded traditional molecular exchange models which are based on passage through single membrane systems, leading some researchers to suggest novel transport mechanisms may be at work (Patrick, 1989; Smith and Smith, 1989). Some researchers have used other analogous biotrophic relationships such as: bean seed coats, lichens, and pathogenic rust and mildew associations for parallels to help understand transport across the biotrophic plant-fungi interface (Smith and Smith, 1990). For example, Ashford et al, (1989) inferred from other biotrophic associations that the apoplastic compartment of the plant-fungi interface was most likely sealed which increases the efficiency of transfer across the interface.

Mechanisms and pathways of energized transfer between symbionts

There are undoubtedly many factors and transport processes involved in nutrient exchange transfer between symbionts (Smith and Read, 1997). Molecular transfer can be reduced to two steps: (i) molecular transfer between symbionts, and (ii) molecular translocation with the fungi. A mechanistic understanding how solutes, both membrane permeant and impermeant are transferred from host plant to fungi across the biotrophic interface is important. The following discussion will begin with an assessment of likelihood that thermodynamically favorable proton and water potential gradients influence molecule transfer across an arbuscule, with an emphasis on water potential gradients, including potential pathways and evidence of water potential assisted transfer. The discussion will also address mechanisms of fungal translocation, again with an emphasis on water potential assisted translocation.

Proton-coupled transport

It has been inferred, from the discovery of at the biotrophic interface that proton-coupled transporters are likely to be involved in CMN-meditated influx and efflux (Smith and Read, 1997). Guttenberger (2000) showed acidification of the shared interfacial apoplastic compartment using acid sensitive dyes. The detection of H^+-ATPases on the membranes of the biotrophic interface led to modeling of proton-coupled influx (Smith and Smith, 1990) of reduced-carbon (hexoses) from plant into the fungus and proton-coupled uptake of phosphate from fungus to plant (Karandashov and Bucher, 2005). Smith and Read (1997) hypothesized that the distribution and presence of membrane-bound H^+-ATPases bordering the interfacial arbuscular matrix indicates active uptake is involved in nutrient transport. More research is needed to elucidate the role of proton-coupled transport mechanisms in relation to the regulation of nutrient transfer across both plant and fungal membranes.

Water potential assisted transport through the biotrophic interface

Evidence suggests water potential driven solute transfer occurs from plant to fungi occurs. Nocturnal transfer of three membrane impermeant tracers from host plant to extraradical AM hyphae was observed (Querejeta et al. 2003), suggesting positive root pressure facilitated transfer from the plant to the fungi. The authors were not able to distinguish if the dyes were released symplastically or apoplastically from the host plant into the biotrophic apoplastic compartment. If the primary site of dye transfer was the arbuscules and the arbuscular apoplastic interface is a sealed compartment lacking continuity with the host cellõs outer cell wall (Ashford et al. 1989) then symplastic efflux into the arbuscular apoplastic interface becomes plausible.

Aquaporins at the biotrophic interface: an expanding role

Understanding of the role of aquaporins, a class of membrane-intrinsic proteins, with the capacity to enhance and regulate hydroconductivity through membranes, continues to expand. In plants, four families of aquaporins have been identified based on their protein sequences (Marjanovic et al, 2005), including plasma membrane proteins (PIPs) and nodulin-intrinsic proteins (NIPs). NIPs are being especially interesting since they are found in plant membranes at the biotrophic interface between the host and symbiotic bacteria. Aquaporins are enriched in areas where high water and solute flux is expected, such as biotrophic interfaces between plants and fungi (Tyerman et al, 2002). In addition to serving as water channels in plants, there is compelling evidence that aquaporins serve as solute channels for glycerol, urea, amino acids or small peptides (Biela et al, 1999; Gerbeau et al, 1999; Krajinski et al, 2000; Tyerman et al, 2002). Furthermore, plant aquaporins appear to possess a highly conserved molecular gating mechanism that is regulated by water potentials (Tornroth-Horsefield et al, 2006). There is also clear evidence that aquaporins play an important role in host plant/AM fungi relations during water stress conditions. Porcel et al, (2005) reported a lower growth rate with antisense PIP mutant NtAQP1 tobacco plants when inoculated with *G. intraracides* during water-stress conditions. Differential expression of PIP genes was reported by Porcel et al, (2006) in lettuce (*B. japonicum*) using AM fungal species, *G. mosseae* and *G. intraracides.* The role of aquaporins in the plant/AM fungal interface is slowly unfolding and will undoubtedly continue to be the subject of intense research. The realization that aquaporins can function as solute channels and are differentially affected by water stress is of particular interest to those involved in molecular interplant CMN transfer studies.

Turgor pressure induced solute efflux into biotrophic interfaces

There is evidence that plant cell turgor pressure triggers symplastic efflux of solutes and ions into the biotrophic interfaces of bean (*Phaseolus vulgaris* L.) seed coats (Patrick 1984, 1989; Zang et al, 2004). Efflux of solutes including ^{14}C-labelled photosynthates and Cl^- in bean seed coats has been shown to be triggered by increasing turgor pressure. There is also evidence that elevated plant cell turgor pressures impede sugar influx (Wyse et al, 1986; Ellis et al, 1992). Although, there is no direct physiological evidence that the turgor state of either plant or fungal membranes affects molecular exchange, largely because the arbuscular morphology is not conducive to direct study, it cannot be ruled out, especially in light that it occurs in other biotrophic associations.

Exo- and endocytosis at the biotrophic interface

It is unknown if the processes of exo- and endocytosis occur at the biotrophic interface. The large demand for plant and fungal membrane during arbuscular formation when membrane surface areas increase 3-10 fold (Cox and Tinker, 1976) vis-à-vis the original plant cell suggests exocytosis occurs and makes is possible for temporal concurrent molecular transfer between symbionts. Endocytosis and exocytosis (Low and Chandra, 1994; Thiel and Battey, 1998) occur in plants and it is likely that endocytosis occurs in yeast (Read and Kalkman, 2003). Interesting, water pressure-induced endocytosis and exocytosis in plant protoplasts was reported by Zorec and Tester (1993). To date, there is no convincing evidence of either exocytosis or endocytosis occurring in AM fungi.

Fungal translocation

Absent of any evidence to the contrary, translocation is believed to occur symplastically within the fungal cytoplasm (Smith and Read, 1997). The aseptate morphology, i.e. the lack of cell walls in all but the smallest diameter AM hyphae, supports relatively high rates of molecular translocation. It is likely that several factors acting in concert drive molecular translocation, yet it has been difficult to discern the contribution of each factor. Cytoplasmic streaming, motile tubules, and diffusion have been observed as factors driving translocation, but have been largely discounted as primary factors because they could not individually account for the observed translocation rates in excess of 20 cm hr^{-1} (Finlay and Read, 1986a) occurring within fungal hyphae nor the observed solute transfer flux occurring between symbionts. It has been demonstrated that water potential can accelerate rates of translocation but was discounted as a primary mechanism of translocation because it could not account for translocation away from the host plant (Smith and Read, 1997).

Cytoplasmic streaming

Initially cytoplasmic streaming was proposed to facilitate translocation of solutes in discrete vacuoles/compartments (Harley and Smith, 1983) within the fungi. This idea evolved from the suggestion by Cox et al (1980) that phosphate-enriched ógranulesô were directionally transported by cytoplasmic streaming. The existence of the phosphate (P) granules has been questioned as being an artifact of histological fixation (Smith and Read, 1997) and P is currently believed to be primarily translocated as polyphosphate, a more soluble form of P. Furthermore, the absence of a loading and unloading system, and questions regarding expected versus observed rates of translocation have effectively discounted cytoplasmic streaming as a principle driver of translocation (Smith and Read, 1995).

Diffusion and motile tubules

Translocation distances and rates driven by motile tubules and diffusion do not reconcile with observed translocation distances exceeding 20 cm nor observed rates of translocation in excess of 20 cm h^{-1} (Finlay and Read, 1986a). Motile tubules have been shown to translocate fluorescent markers over small distances in growing fungal hyphae

(Shepherd et al, 1993). The observed rates of motile tubule translocation precludes it's consideration as the principle driver of fungal translocation. In the case of diffusion, fungal morphology makes the movement over longer distances by diffusion alone inefficient. Skinner and Bowen (1973) reported rates of translocation of phosphate several orders of magnitude higher than if by diffusion alone.

Water potential-driven translocation: Negative pressure across the biotrophic interface

The extent of water potential driven mass flow as a factor facilitating molecular translocation has been questioned, even though it could potentially account for the observed translocation distances and rates. Researchers demonstrated actively transpiring host plants had accelerated rates of translocation toward the transpiring plant (Cox and Tinker, 1976; Cooper and Tinker, 1981). Skinner and Bowen (1973) found the rate of phosphate translocation increased 2-3 folds toward the end of an excised EMF mycelium submersed in an osmoticum. Pearson and Read, (1973) found the distance of ^{32}P translocation in the fungi significantly increases when associated with a transpiring plant. Finlay and Read (1986a; 1986b) discounted bulk flow as a principle driver of translocation because if evapotranspirational effects largely dominated the transport process the flow would take place in a unidirectional manner towards the transpiring host plant. Translocation of phosphate and reduced carbon in ectomycorrhizal fungi was bi-directional towards the host plant and towards the terminal mycelia fans contradicting the predicted bulk flow movement (Finlay and Read, 1986a; 1986b). Smith and Read, (1997) stated, mass flow (toward the transpiring plant) does not allow for the bi-directional translocation of nutrients which certainly occurs in the mycelium as a whole. These conclusions ignore the possibility that positive root pressure can explain translocation away from the host plant. The underappreciated outcome of aforementioned translocation studies was the results demonstrate that the existence of hydraulic pathway across the biotrophic interface, one that was not arrested at the biotrophic interface.

Water potential driven translocation: Positive pressure across the biotrophic interface

Water potential induced root pressures diurnally fluctuate between positive and negative. When transpiring, a plant creates a negative pressure as water vapor escapes when CO_2 enters. When stomata are closed, osmotically generated positive root pressure develops by the influx of water into roots down its potential gradient. This phenomenon can be readily observed as guttation in leaves, hydraulic lift in roots, and sap exudation in cut tree stumps (Kramer and Boyer, 1995). Querejeta et al, (2003) showed nocturnal transfer of three water soluble dyes from a well watered oak root into water-stressed AMF hyphae demonstrating hydraulic conductivity from root to fungi. The movement into the distal tips of AMF hyphae provides evidence for water potential driven influx and translocation of water and water-soluble dye into and away from the host plant.

Interplant transfer studies

Applying source/sink theory to facilitate interplant transfer

Temporal and spatial source/sink gradients are believed to be the primary factor influencing rate and amount of molecular transfer within a CMN. There is a general consensus that nutrients move within a CMN from an area of higher concentration (source) toward an area of lower concentration (sink), over time. Is has been a common practice to develop experimental protocol with the aim of forming a molecular source and sink to study molecular transfer via mycorrhizae (Simard and Durall, 2004). The formation of a source and sink varies depending on the nutrient. For soil derived nutrients such as phosphorus, a spilt pot design has been used; with a donor plant grown in a P enriched environment and the receiver in a depleted environment. In N transfer studies researchers have created a source/sink model using an N-fixing legume as the donor plant and a non N-fixing receiver (Haystead et al, 1988; Hamel and Smith, 1992). Laboratory or greenhouse carbon transfer studies commonly create a carbon sink by either shading or de-stemming a receiver plant and measure transfer using carbon isotopes (Waters and Borowicz, 1995; Fitter 1998, 1999; Robinson and Fitter, 1999; Fitter 2001; Pfeffer et al 2004). In the several water relations studies, the water source/sink model was applied to an óincompleteô, not ówholeô CMN, i.e. a host plant and corresponding extra radical AM hyphae only (Auge, 2001). A physical barrier spatially separating the host plant from distal extra radical hyphae of fungi creating two distinct compartments allowing either the plant or fungal compartment to function as the water source or sink has been used to study water relations (George et al, 1992; Querejeta et al, 2003). Methods for measuring water transfer include calculating soil water content (George et al, 1992), or using fluorescent tracers (Querejeta et al, 2003) as a proxy for water transport.

Phosphorus transfer

Phosphorus (P), is a soil-derived nutrient and the primary transfer pathway is from fungi to plant. The pathway begins with fungal uptake, then P translocation toward the host plant and transfer across the biotrophic interface into the host plant. There is also strong evidence indicating that interplant P transfer is enhanced in the presence of AM fungi (Chiariello et al, 1982; Francis et al, 1986; Fisher-Walter et al, 1996). Fisher-Walter et al, (1996) reported enhanced P transfer between tallgrass prairie plants, and at significantly greater distances than had previously been reported, suggesting a possible mycorrhizal pathway. The 2 wk exposure period combined with lack of a fungicide affect on transfer, prevent ruling out an indirect transfer pathway. It has been argued that interplant P transfer does not occur by an entirely direct transfer pathway (Smith and Read, 1997). The difference between a direct and indirect P transfer pathway is centered on fungal P acquisition. If the fungus scavenges P from the senescing roots of the donor plant then the pathway is considered indirect. If the fungal P uptake occurs from a healthy root then the pathway is direct.

Nitrogen transfer

Interplant transfer of nitrogen has been shown to be enhanced by the presence of AM fungi (Haystead et al, 1988; Hamel and Smith, 1992; Tobar et al, 1994; Martensson et al, 1998; Moyer-Henry et al, 2006). Hamel and Smith, (1992) showed that labeled ^{15}N was transferred between soybean and corn planted in the same row over a growing season. Haystead et al, (1988) reported ^{15}N transfer between clover (*Trifolium repens* L.) and a grass (*Lolium perenne* L.). Net transfer on ^{15}N was observed between nodulating and non-nodulating soybeans and nodulating (*Glycine max*) and mycorrhizal weeds but not non-mycorrhizal weeds (Moyer-Henry et al., 2006). The length of the exposure period and the lack of a direct visible transfer pathway in all of these studies have made it difficult to distinguish if N was transferred directly through AM fungi or entered the soil pathway at some point.

Water transfer: temporal source/sinks controlled by the host.

There has been an extensive amount of research which indicates mycorrhizal symbiosis improves host plant water relations during water stress conditions (Auge, 2001). Mycorrhizal colonization increases stomatal and hydraulic root conductance, transpiration rate, and plant growth (Auge, 2001). Attempts to determine if the fungi are directly responsible for improved water relations during water-stress have indicated AM fungi increase water use efficiency, but there is little evidence the fungi directly augments the host plant water supply (Auge, 2001).

Studying direct fungal water uptake of a mycorrhizal root system to measure water uptake from a spatially separate root-free compartment that either contains hyphae or not, has been a commonly used method to calculate net water uptake (Auge, 2001). Bryla and Duniway, (1997) reported no significant differences in water uptake between mycorrhizal and non-mycorrhizal roots of safflower or wheat during water stress. In a similar study using couchgrass (*Agropyron repens*) and white clover (*Trifolium repens*) George et al, (1992) reported significant transfer of P and N from a hyphal compartment, but no difference in water uptake under well-water and water-stressed conditions whether hyphae were present to not. Based on these findings the authors of both studies concluded no significant net water uptake and transfer occurred. The conclusion may be erroneous if the host plant functioned as the water source, and water source/sink gradient are temporal. If the host plant functions as the water source during water-stress conditions then overall net transfer from the hyphae compartment toward the host should be expected. Depletion of water from a hyphae-only compartment should not be expected even when temporal net water movements occur. Temporal water source/sinks would favor net water movement toward the fungi at night and net water movement toward the host during the day, resulting in no net spatial transfer between compartments. In another experiment using a split pot design, Auge, (1994) reported the mycorrhizal *Zea mays* roots systems dried the soil more slowly than non-mycorrhizal root systems. This is the expected outcome, provided the well-watered host plant acted as the primary water source for the fungus. Direct evidence of a host plant acting as the water source was provided in a study by Querejeta et al (2003) who observed the nocturnal movement of dyes from the host plant toward the distal ends of the hyphae and even into the water-stressed soil adjacent to the hyphae. It is important to mention that water transfer experimentation has been restricted to mycorrhizal roots systems, not whole CMNs, unlike other nutrients. Future research using biologically inactive tracer molecules, serving as a proxy for interplant water transfer may be useful.

The carbon transfer debate

Carbon transfer is the most intensely studied area of CMN-mediated nutrient transfer. Conflicting research results regarding interplant C transfer have created a debate whether interplant net carbon transfer occurs. There are results which suggest that interplant transfer of C does not occur and that C fails to efflux into the receiver plant tissue (Waters and Borowicz, 1995; Fitter 1998, 1999; Robinson and Fitter, 1999; Fitter 2001; Pfeffer et al 2004) and contradictory results which support net interplant C transfer (Smith and Smith 1990; Simard 1997; Graves 1997; Martin et al, 1997; Lerat et al 2002; Carey et al, 2004). Many of these studies assume primary mechanism of C movement within a CMN is C gradients, which will move C from a source, an area of high concentration, towards a sink, an area of lower concentration, over time. Consequently several C transfer studies used an intact donor plant as the source and a de-stemmed or shaded receiver plant as a sink to facilitate C transfer.

Carbon influx, translocation, but no efflux: No net carbon transfer

Several studies indicate C influx and translocation into and through fungal hyphae but no efflux into the receiver plant tissue (Graves 1997; Fitter 1998, 1999; Robinson and Fitter, 1999; Fitter 2001; Pfeffer et al 2004). A study using *Cynodon dactylon* (C_4) and *Plantago lanceloata* (C_3), as both donor and receiver plants, indicated C remained within the fungal tissue in the receiver root (Fitter et al, 1998). These results indicate influx into and translocation across the CMN of labeled carbon but failure of C to efflux into the receiver plant tissue.

Carbon influx, translocation, and efflux: Net carbon transfer

There is evidence in favor of interplant C transfer. Hirrel and Gerdemann (1979) showed evidence of enhanced C transfer between onion plants when infected with mycorrhizal fungi. Evidence of interplant C transfer between *Festuca idahoensis* and *Centaurea maculosa* was reported by Carey et al, (2004). A study by Bidertondo et al, (2002) found three species of AM fungi present within the roots *Arachnitis uniflora*, an epiparasitic (mycoheterotrophic) plant found in sub-Antarctic forests in Argentina. The authors concluded the epiparasitic plant depends on AM fungi for C transfer from the donor plant since only AMF colonized both plants. This study in particular indicates of ecologically significant net flow of reduced C between plants linked to a CMN.

Could an opposing water source/sink gradient be the cause of the conflicting results?

The experimental design of the transfer studies showing no interplant C transfer used methods designed specifically to produce a carbon source/sink relation between donor and receiver plant to facilitate interplant C transfer. The downside of forming a C source/sink by shading or de-stemming is that it also alters the water relations between the interconnected plants. The studies have ignored the possibility that diurnal plant-derived water pressure gradients may facilitate molecular transfer/translocation within a CMN. In fact, by applying the same source/sink template to water transfer, an alternative explanation of the results can be made, namely that water source/sinks affected movement C source between linked plants. In fact, in several studies (Graves 1997; Fitter et al 1998, 1999; Robinson and Fitter, 1999; Fitter 2001) the receiver plant stem was removed to produce a carbon sink. Plants without stems would presumably have little if any transpiration and develop positive root pressure assuming soil water supply is adequate. Thus, the receiver root pressure would act as a water source and the donor plant the sink during active donor transpiration, effectively creating a water source/sink in direct opposition to the C source/sink. Elevated plant cell turgor pressures have been reported to impede sugar influx rates (Wyse et al. 1986; Ellis et al. 1992). Waters and Borowicz (1994) found carbon moved away from the clipped receiver plant toward the unclipped donor plant, when linked by hyphae, opposite of what was expected according to source/sink theory when applied only to carbon. They suggested the movement away from the clipped plant was due to increased labile carbon in the root, released by clipping of stem. Another possibility is that elevated turgor in the receiver roots triggered the release of carbon similar to the phenomenon reported by Patrick et al (1989) in bean seed coats.

Evidence of water potential assisted transfer in AM fungi

Plant-derived water pressures exist; and there is also evidence that hydraulic conductivity occurs between symbionts and is not arrested at the plant/fungal interface (Pearson and Read, 1973; Skinner and Bowen 1973; Cox and Tinker, 1976; Querejeta et al, 2003). Therefore, it seems reasonable to expect that the symbiosis has evolved strategies to utilize water potentials for their mutual benefit when appropriate. In addition to fluorescent tracer studies, there is circumstantial evidence suggesting other solutes can be transferred with a CMN along with water. Querejeta et al, (2003) observed only nocturnal movement of membrane impermeant dye from oak roots to hyphal tips. The dye was also observed in the soil around the hyphal tips. These observations support the idea that novel biotrophic transfer mechanisms, facilitated by water potential gradients may be responsible.

Summary

Most interplant carbon transfer studies have proceeded on the assumption that creating a carbon source and a carbon sink will drive interplant carbon transfer through a CMN. Often either the stem of the receiver plant is removed (Fitter et al, 1998) or is it shaded (Francis and Read, 1984), to create a carbon sink in the receiver plant relative to the donor plant (source) to facilitate interplant transfer. The removal of all the above ground biomass of the receiver plant to create a carbon sink also has the unintended consequence of creating water pressure gradients which may inhibit molecular transfer from donor plant to receiver plant. Designing experiments which, reduce transfer time, allow for visualization of transfer, and utilize appropriate tracers while applying the same source/sink model used in other CMN transfer studies to water, may help reconcile the seemingly contradictory results found in interplant CMN transfer and water uptake research.

Chapter II: **Forming soil free 3-D arbuscular mycorrhizal networks with *Glomus intraradices* using Illinois bundleflower.**

INTRODUCTION

It has been estimated that 70% of all terrestrial plant species live in symbiosis with mycorrhizal fungi. Mycorrhizal fungi are capable of forming extensive extraradical (ER) hyphal networks emanating from the host plant root into the rhizosphere (Francis et al., 1986). In laboratory studies hyphae been shown to be capable of simultaneously infecting neighboring plant roots, whereby forming an ER mycorrhizal network (Simard and Durall, 2004).

Extraradical AM hyphal networks are intrinsically difficult to study without physically disrupting or physiologically altering network development. Soil and soil-free culture systems have been developed and successfully used to overcome some of these inherent challenges, yet each system has limitations. These systems include: (i) root observation chambers (Friese and Allen, 1991); (ii) a two-dimensional culture system; (Giovanetti et al. 2001, 2004) and (iii) transformed and non-transformed root-organ cultures (Mosse and Hepper, 1975; Mugnier and Mosse, 1987; Declerck et al. 2004).

Root observation chambers with soil have been used to visualize the developmental phenology and architecture of ER hyphal, yet viewing was limited to only hyphae growing directly adjacent to the observation chamber glass (Friese and Allen, 1991). The two-dimensional culture system (Giovannetti et al. 2001, 2004) proved effective for studying anastomosis at hyphal contact points, but prevents three-dimensional hyphal growth. Root-organ culture systems have proved useful for studies of AM fungal ER development, including: morphology of over twenty AM fungal species (Fortin et al. 2002); sporulation (Declerck et al. 2004); and environmental nutritional effects on ER hyphal development (Fortin et al. 2002; Diop, 2003). Root-organ culture systems are best utilized for understanding AM fungal interactions with root systems rather than whole plants, since root-organ cultures lack a functioning stem.

Several areas of AM fungal research could potentially benefit from a system that reliably creates three-dimensional soil-free ER hyphal networks using whole plants. These research areas include: general ER AM fungal network architecture; host plant compatibility; leguminous nodule hyphal linkages; direct nutrient sequestration from senescing roots; and interplant molecular transfer.

The objectives of this study were: (i) to create a novel system that reliably forms three-dimensional soil-free ER networks using indigenous sources of AM fungi; and (ii) to describe aspects of ER fungal development in the system, i.e. hyphal architecture, sporulation, hyphae/nodule interactions, and hyphal linkages.

MATERIALS AND METHODS

Fungal source. ð Soil (well-drained Waukegan silt loam) from the upper 20 cm was collected 10 Apr 2006 from a field with a four-year leguminous cover-cropping history at the University of Minnesota Outreach, Research, and Education Park near Rosemount, MN, USA. The finer root fragments were left in the unsterilized soil to facilitate AM fungal infection of host plants. The soil was mixed with an equal amount of sterilized sand to increase soil porosity without jeopardizing inoculation effectiveness. The mixed soil was placed in 3.8 L (1 gal) pots.

Host plant inoculation. ð All host plants, with the exception of Johnson grass ([*Sorghum halepense*] seedlings collected from the soil site) were grown from seed for 6-8 wk to allow AM fungal inoculation from soil propagules. Plants were removed from the soil, gently rinsed, and paired with another plant of similar stature. Fungal root colonization was determined by staining the lateral roots (McGonigle et al. 1990).

Nitrocellulose sandwiches. ð A piece of nitrocellulose (NitroBind, 0.45 um) paper was folded over the roots of two inoculated plants (Fig. 1) forming a nitrocellulose sandwich. Standard fiberglass window mesh (18 × 16) rectangles (3.5 cm × 6.5 cm) were used to structurally reinforce nitrocellulose sandwich. Sandwiches were carefully closed using one or two small metal paper clips to prevent damaging the roots. The sandwiched pairs were placed in either coarse or medium grade vermiculite and allowed to grow for 4-8 wk to ensure adequate external hyphal development. The transplants were acclimatized for 1 wk in a misting room (8 s mist every 8 min) before transferring to another greenhouse (23-33 C; natural light). Four sandwiches were planted per pot (1.9 L).

*Primary host plant selection.*ð Seventeen sandwiches; 3 Illinois bundleflower (*Desmanthus illinoensis*-IBF) pairs, 3 red clover (*Trifolium pratense*)ï Illinois bundleflower pairs, 3 alsike clover (Trifolium hybridium) pairs, 3 soybean (*Glycine max*) pairs, 3 corn (*Zea mays*) pairs, and 2 Johnson grass (*Sorghum halepense*) pairs were screened for i) hyphae linkages ii) a suitable rate of root growth which did not out grow the nitrocellulose sandwich, and iii) trouble-free root identification i.e. which root belonged to which plant.

Viewing ER networks. — After 4-8 weeks the sandwiches were gently removed and rinsed of excess vermiculite. The nitrocellulose sandwich was opened gently to minimize damage to extraradical hyphae. Digital imaging of extraradical hyphae was done using a Leica FluoCombi III stereomicroscope (8× ï 10×). In

Determining hyphal continuity with Phorwite. — Six weeks after transplantation, 5 nitrocellulose sandwiches, with paired IBF plants were exposed to Phorwite BRU Liquid ™ (Bayer) exposure. The sandwiches were gently removed and rinsed of excess vermiculite. The paired plant root were separated and submerged in two 37 mL glass vials containing either (i) 1% $^{w/v}$ Phorwite or (ii) a 15% 10 000 MW polyethylene glycol (PEG), in distilled water. A15% PEG solution produces osmotic potential of -0.25 ±.03 MPa (Caston Guay, thesis) simulating drought stress conditions (Kramer and Boyer, 1995). The 16 h exposure period consisted of 4 h light: 8 h dark: 4 h light.

Molecular identification using ER hyphae. — Hyphae were collected for polymerase chain reaction (PCR) amplification either using with a forceps or by rinsing the nitrocellulose paper with distilled water and filtering with a 10 *um* nylon mesh. Samples were stored in distilled water at ï 20 C prior to PCR amplification. Each sample was ground in liquid N by mortar and pestle and DNA was extracted using a DNeasy Plant Mini Kit (Quigen). PCR amplification consisted of a single round of PCR using the primer pair AM1 (Helgason et al, 1998) and NS31 (Simon et al. 1992) in 40 *ul* reactions including 4 *ul* 10x CoralLoad PCR buffer (Taq DNA polymerase, Quigen), 2 *ul* each primer (1 *um* conc.), 4 dNTPs (1 um conc.), 0.4 *ul* Taq, 2 *ul* DNA. Following a hot start, thermal cycling included an initial denaturation step of 94 C for 2 min, [92 C for 30 sec, 57 C for 15 sec, 72 C for 30 sec] for 35 cycles, and 72 C for 5 min. PCR products were checked on 0.8% agarose gel with a 100bp ladder for a fragment check with the remaining product purified via a PCR CleanUp Kit (Promega). The clean product was directly sequenced with the same primers on an automated capillary elctrophoretic DNA sequencer ABI 3130xl using Big Dye Terminator v.3.1. The sequence results were analyzed using nBLAST (Dumontier and Hogue, 2002) in the NCBI database and aligned using CLUSTAL W (Thompson et al, 1994).

RESULTS

The culturing system formed root-to-root hyphal linkages, root-to-nodule linkages, spores, and suitable quantities of ER hyphae for DNA amplication and molecular sequence analysis.

Plant screening. — Extraradical hyphal bridges were viewed in all screened sandwiches; 3 Illinois bundleflower (*Desmanthus illinoensis*) pairs, 3 red clover (*Trifolium pratense*)ï Illinois bundleflower pairs, 3 alsike clover (*Trifolium hybridium*) pairs, 3 soybean (*Glycine max*) pairs, 3 corn (*Zea mays*) pairs, and 2 Johnson grass (*Sorghum halepense*) pairs. Sandwiches with more lateral roots had more ER hyphae. The rapid root growth of corn and soybean made viewing difficult. The fibrous root systems of both red and alsike clovers created robust hyphal linkages yet the high root density made root identification challenging. Both Johnson grass and Illinois bundleflower had suitable rates of root growth, and easily identifiable roots. Illinois bundleflower, a legume, was chosen as the principal plant for further system research and development.

Extraradical hyphal development and morphology. ð Three-dimensional ER hyphal networks were observed in all sandwiches although the robustness of these networks did vary. Sandwiches with fewer lateral roots had less vibrant networks. Physical detachment of the hyphae was limited to hyphae directly attached to the nitrocellulose paper. Angular dichotomous branched ER hyphae were observed in the three-dimension area of the sandwiches. A web-like conformational structure was observed in sandwiches with the highest densities of ER hyphae (Fig. 2).

Extraradical hyphal and mycelia linkages. —The system produced ER hyphae linkages within the same root system and between two root systems. Intra- and interspecies linkages were observed.. All sandwiches produced observable hyphal linkages regardless of the pairing combination although the extent of hyphal linkage did vary. Sites of more extensive hyphal linkages occurred at: lateral root tips; between apparently healthy and senescing roots (Figs. 3 & 4), and any nodule (Fig. 5). Development of extensive and/or chord-like mycelium linking an apparently dying root (Note: roots were darkened with fragmented root tips and/or associated with a defoliated stem) with a healthy root was common using the system. These linkages occurred between two separate root systems (Fig. 3) and within a single root system (Fig 4).

Nodules linkages. — Nodules were one of the best sites to observe ER hyphae (Figs. 5&6). Hyphal linkages were observed between the nodule and: (i) a root of the partner plant (Fig. 6), (ii), a different root of the same plant, or (iii) a site above or below nodule on the same root.

Sporulation. —It was common for IBF plants to defoliate within 14 days of sandwich creation/transplantation. Spores were observed in all five sandwiches when one of the two IBF plants in the sandwich defoliated at six weeks after transplantation. Spores were observed externally associated with defoliated IBF roots (Fig. 7), organic matter within the sandwich, or attached to the nitrocellulose paper (Fig. 8). Spores were often viewed in groups of 10-20 and had morphology consistence with *Glomeraceae* (Fig. 7). Visible accumulation of Phorwite in the spores associated with the defoliated plant root system occurred within 16 h of dye exposure (Fig. 7), or within spores associated with organic matter indicating the existence of hyphal hydraulic continuity between the spores and the foliated host plant. Spores and arbuscular-like structures (highly branched hyphae) were observed associated with the nitrocellulose paper (Fig. 8) using a fluorescent dye. It is likely they were present on the dormant root surface or in the organic matter just not readily identifiable.

Molecular identification of indigenous fungi. — Sequence analysis of the 18 S rRNA gene isolated and PCR amplified directly from ER hyphae identified the indigenous fungi as *Glomus intraradices* in 7 of the 8 samples tested. Sequence similarity ranged between 99-100% (Table I).

DISCUSSION

The ER hyphal morphology was similar to previous reports of ER AM hyphae (Friese and Allen, 1991; Bago et al, 1998; Smith and Read, 1999). Where the flaps of nitrocellulose paper constricted hyphal growth within a two-dimension area, hyphal architecture was similar to previous accounts using other soil-free systems (Bago et al, 1998; Giovannetti et al, 2001, 2004).

Extensive or chord-like mycelia emanating from root fragments (Diop, 2003) and forming linkages with healthy roots are capable of inoculating healthy roots (Friese and Allen, 1991). The overall robustness of the mycelia linkages between the roots of the same plant (Fig. 4) seems excessive for propagation purposes alone. It would not be surprising if future research reveals that the mycelia are involved in nutrient sequestration.

Since first reported by Ames et al (1983), interplant transfer of nitrogenous compounds has become an active area of AM fungal research. This system may be a valuable tool used to monitor interplant transfer of nitrogenous compounds, or any molecular compound, over relatively short time periods, i.e. hours, since the ER hyphal pathway is easily visible, and reliably generates interplant hyphal linkages.

This system has proved to be effective at observing certain facets of AM fungal sporulation such as arbuscular-like structures, hypothesized by Bago et al (1998) as ER sites of nutrient uptake supporting spore development, or studying if environmental factors, such as chemical cues, initiate site specific sporulation. The results suggest defoliation may trigger site-specific sporulation on the surface of dying roots, similar to results reported by de Souza and Declerck, (2003) who observed sporulation on the surface of older roots using a root-organ culture system, or at nutrient enriched sites (Fig. 6). An interesting phenomenon revealed by the system was that the spores maintained hyphal hydraulic continuity with the foliated plant within the sandwich (Fig 6). This may indicate that *Glomus intraradices* selectively sporulates on dying roots or at nutrient enriched sites, which would be an effective reproductive strategy to sustain itself within plant communities. It must be noted that the spatial constraints of the system may have biased the results and that additional research will be needed to determine if selective sporulation is an actual phenomenon.

Molecular identification of an AM fungal species is often complicated by simultaneous infection with multiple species or successfully extracting enough DNA from the plant tissue. DNA extraction and amplification directly from ER hyphae overcomes problems with simultaneous infection and the absence of plant tissue simplifies DNA extraction.

Several systems have been used successfully to study ER development and function, yet limitations remain. This system provides an effective method to reliably produce three-dimensional ER networks between whole plants in the absence of soil. The system may benefit researchers interested in ER architecture and function, interplant molecular transfer research, sporulation, or AM species identification.

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Table 1.
Partial 18S rRNA sequences from external hyphae.

Sandwich #/ primer/collection method***	*Most similar isolate(s) from Genbank*	*(% sequence similarity using BLAST)*	*GenBank accession numbers*
S1/AM1/*ff*	*Glomus intraradices* stain MUCL	(99%)	AJ852526
	Uncultured *Glomus sp.* F9AGMyc61	(99%)	EF041068.1
S1/AM1/*rf*	*Glomus intraradices* 18S rRNA gene	(100%)	X58725.1
	Uncultured *Glomus sp.* F16AGMyc75	(100%)	EF041059.1
S2/AM1/*ff*	*Glomus intraradices* 18S rRNA gene	(99%)	X58725.2
	Uncultured *Glomus sp.* F16AGMyc75	(99%)	EF041059.2
S2/AM1/*rf*	*Glomus intraradices* 18S rRNA gene	(99%)	X58725.3
	Uncultured *Glomus sp.* F16AGMyc59	(99%)	EF041059.3
S3/NS31/*ff*	*Glomus intraradices* stain MUCL	(99%)	AJ852526
	Uncultured *Glomus sp.* F16AGMyc61	(99%)	EF041052.1
S3/NS31/*rf*	*Glomus intraradices* stain MUCL	(98%)	AJ852526
	Uncultured *Glomus sp.* F9AGMyc61	(99%)	EF041068.1
S4/NS31/*ff*	*Glomus intraradices* stain MUCL	(99%)	AJ852526
	Uncultured *Glomus sp.* F9AGMyc67	(99%)	EF041068.1

* two sets of primers AM1 and NS31 targeted to 18S rRNA region
** collection of the hyphae was by fine forceps (ff) or rinsing and filtering (*rf*)

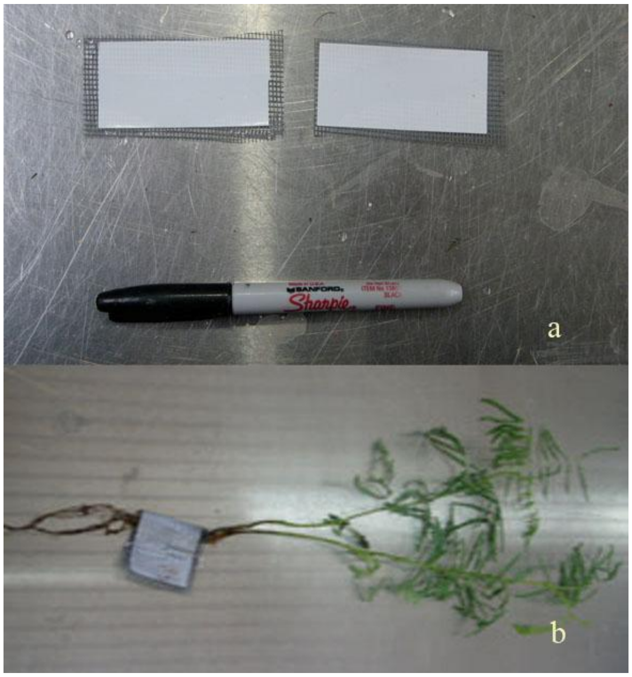

Figure 1. (a) Nitrocellulose (3 cm × 6 cm) with fiberglass mesh for reinforcement (b) nitrocellulose sandwich with two IBF plants secured with two paper clips.

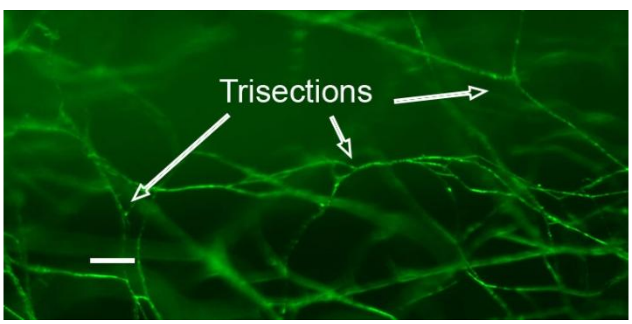

Figure 2. Three dimensional ER hyphal network formed between two healthy IBF root systems. Trisections formed by angular dichotomous hyphal branching. Scale Bar = 200 *u*m.

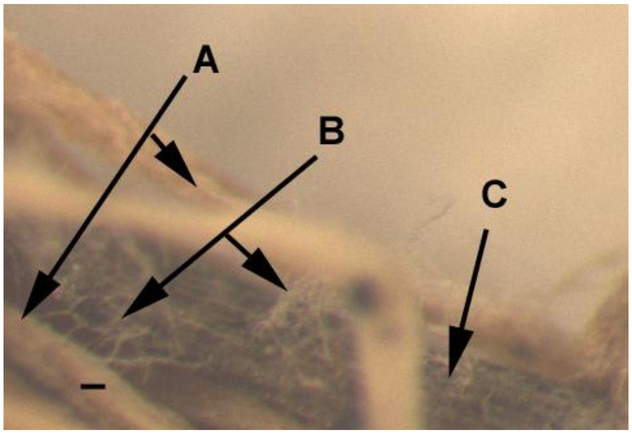

Figure 3. Healthy lateral roots (A) linked by hyphae (B) to decaying (C) IBF roots. Scale bar = 300 um.

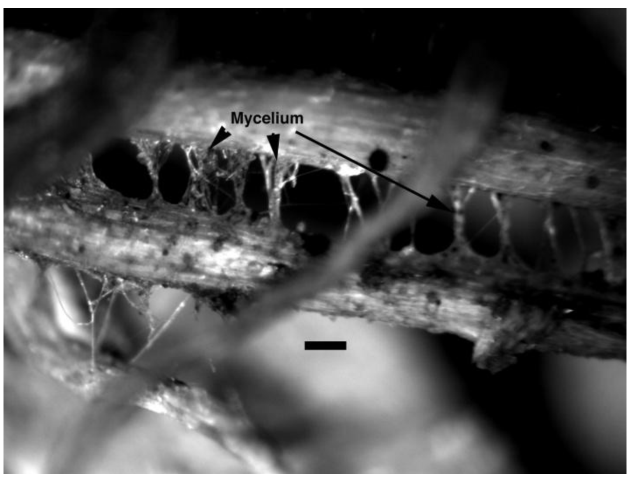

Figure 4. Johnson grass (*Sorghum halepense)* root system. Micrograph shows a healthy root (above) linked by mycelium to a decaying root (below). Scale bar = 300 um.

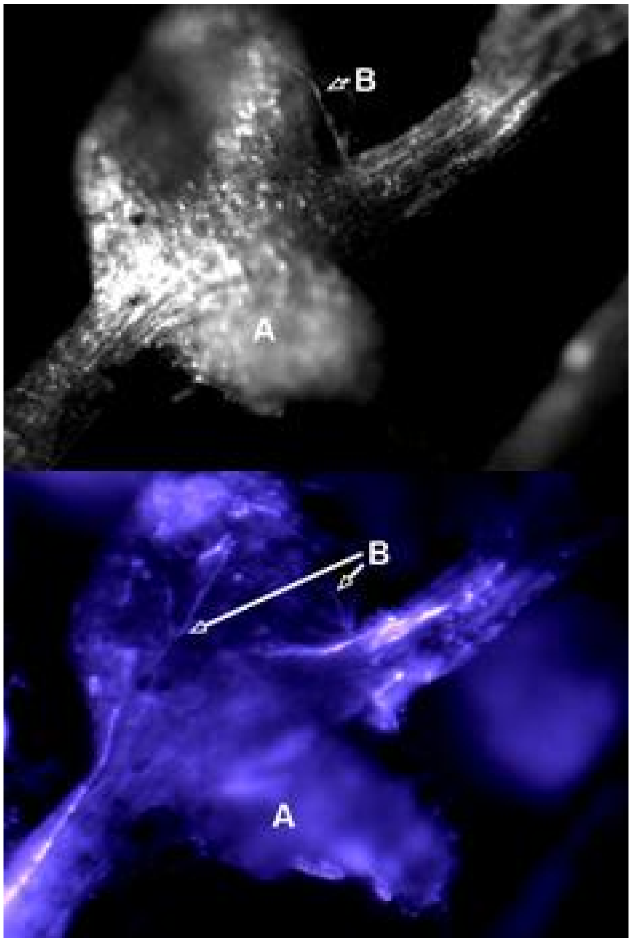

Figure 5. A micrograph of a nodule (A) with ER hyphae (B) from the root system of a defoliated IBF plant using normal light (above) and *UV* light (below). Accumulation of phorwite in the ER hyphal (B) indicates hydraulic hyphal continuity between a foliated donor IBF plant and the defoliated IBF plant. Micrograph was taken less than 20 hrs after dye exposure.

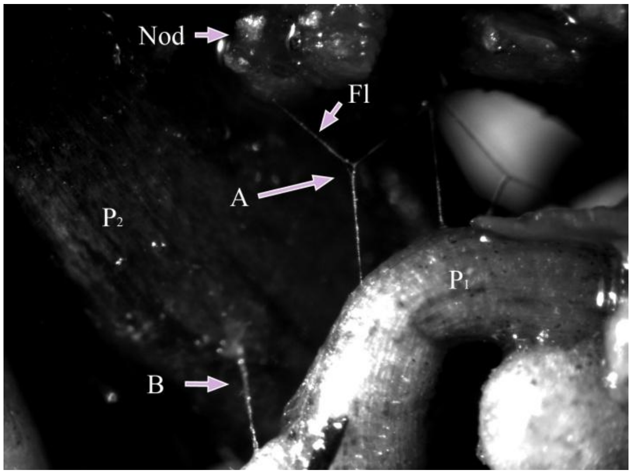

Figure 6. The lateral root (P_1) of an IBF plant with two hyphal links (A & B) to a taproot of the another IBF plant (P_2). Hyphal linkage (A) between the lateral root (P_1) and a nodule primordial (Nod) on the taproot (P_2). Red autofluorescence was observed in the hyphae (Fl) using normal light.

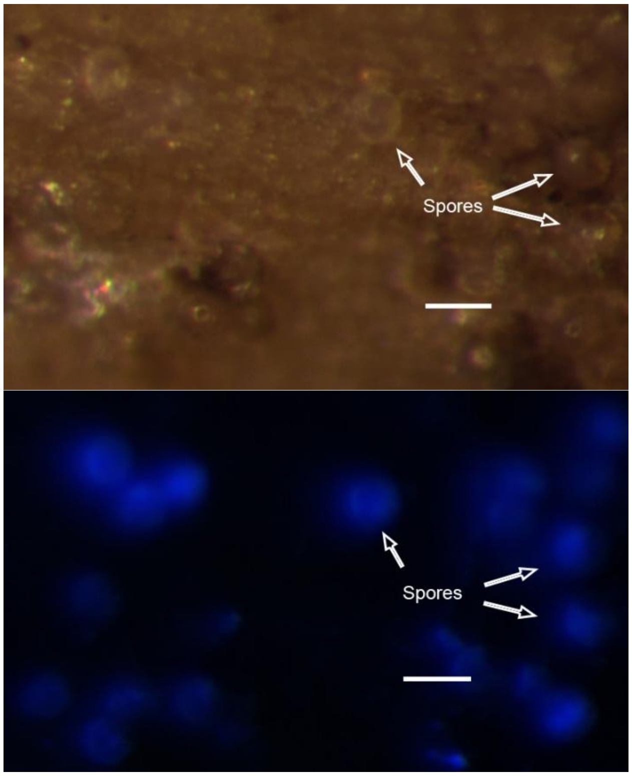

Figure 7. AM fungal spores on the epidermis of an IBF root using normal light (above) and *UV* light (below). Phorwite readily visible in the spores. Scale bar = 300 *u*m.

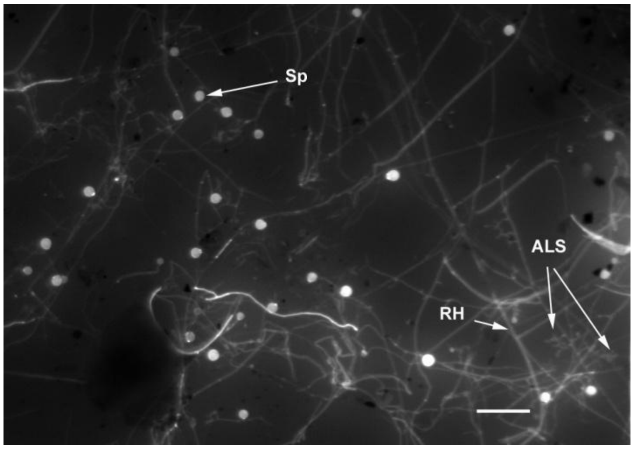

Figure 8. Morphological features of ER mycelium visualized with phorwite. Spores (Sp), runner hyphae, runner hyphae (RH) and highly branched arbuscule-like structures (ALS). Scale bar = 200 *u*m.

Chapter III: Water-stress facilitated direct interplant arbuscular mycorrhizal transfer using *Desmanthus illinoensis*.

INTRODUCTION

When two or more root systems are linked by mycorrhizal hyphae a common mycorrhizal network (CMN) exists (Simard and Durall 2004). Evidence suggests that arbuscular mycorrhizal (AM) hyphae forming a CMN, serve as conduits for direct interplant solute transfer. Enhanced CMN-mediated transfer of nitrogen (Jin et al. 2005), phosphorous, and carbon (Watkins et al. 1996; Lerat et al. 2002; Carey et al. 2004), has been reported. In the case of AM elemental carbon transfer, a controversy exists. The results of several studies indicate little to no interplant carbon transfer occurs (Waters and Borowicz 1994; Watkins et al. 1996; Graves et al. 1997; Fitter et al. 1998). The carbon remains in the fungal tissue and is not transferred in any significant amount to the receiver root. Resolution of this debate may occur by re-examining the methods used to facilitate interplant transfer in carbon transfer studies.

Temporal and spatial source-sink gradients are broadly believed to influence the direction and velocity of solute transfer in a CMN (Francis and Read 1984; Read et al. 1985; Finlay and Read 1986; Simard and Durall 2004). Creating source-sinks gradients between linked plants has been a common experimental technique used to facilitate and study interplant solute transfer, yet in some instances, e.g. carbon transfer studies, their creation also creates an opposing water source/sink in direct opposition to the carbon source/sink.

The importance of water potential driven mass flow within AM hyphae has been questioned (Smith and Read, 1997), even though plant transpiration has been shown to facilitate the rate of translocation within fungal hyphae towards the transpiring host plant (Pearson and Read, 1973; Pearson and Tinker, 1975; Cooper and Tinker 1978, 1981).

Reports of bi-directional translocation of tracer molecules (Finlay and Read, 1986) within hyphae has led researchers to question if water potential driven mass flow is a significant factor driving interplant transfer since it cannot account for the movement of the tracer away from the host plant (Smith and Read, 1997). Actually, bidirectional water potential driven movement should be expected if (i) a host plant diurnally fluctuates between positive (non-transpiring) and negative (transpiring) water pressures and (ii) hydraulic pathway exists across the plant-fungal biotrophic interface. While transpiring, a plant generates a negative water potential gradient favoring bulk flow toward the plant and when transpiration ceases the water potential becomes more positive favoring bulk flow away from the plant toward the fungi provided a hydraulic pathway exists between plant and fungi.

There is convincing evidence supporting the existence of hydraulic continuity across the plant-fungi biotrophic interface. Results of several studies show water pressure is not arrested at the perioarbuscular interface since negative plant water pressure affects the direction and rate of translocation within the fungi (Pearson and Read, 1973; Pearson and Tinker, 1975; Cooper and Tinker 1978, 1981). There is also evidence that positive host plant water potential, developed at night, facilitates translocation away from the host plant into the fungi and transfer of marker molecules out of the fungi into the soil (Querejeta et al. 2003).

The goals of this study were to determine (i) if water potential gradients facilitate transfer of a dye between plants linked by mycorrhizal hyphae, by creating water potential gradients which favor interplant transfer and (ii) using a procedure to visually observe the hyphal transfer pathway and specifically to identify the site the dye enters into the receiver plant tissue.

MATERIALS AND METHODS

Fungal source. ð Soil (well-drained Waukegan silt loam) from the upper 20 cm was collected April 2006 and 2007 from a field with a four-year leguminous cover-cropping history at the University of Minnesota Outreach, Research, and Education Park near Rosemount, MN, USA. The finer root fragments were left in the soil to facilitate indigenous AMF inoculation of host plants.

Illinois bundle flower inoculation.— Illinois bundle flower ([IBF] *Desmanthus illinoensis*) was grown from seed at a density of 25-35 seeds per pot (3.8 L) in a greenhouse. A 16 h light cycle was maintained throughout the entire experiment cycle including grow out and testing (April-Sept) in 2006 and 2007. Supplemental light was used when ambient radiation fell below 500 Wm^2. Temperatures ranged between 21-35 C and relative humidity ranged between 35-92 %. The collected soil was diluted at a 1:1 ratio with sterilized sand to increase soil porosity. Plants were grown for a period of 6-10 wk to ensure indigenous AM fungal inoculation. Colonization of the lateral roots was confirmed by using a staining procedure developed by McGonigle et al (1990).

Nitrocellulose sandwiches and mycelium development.— Between 6-10 wk of age the host plants were i) removed from the soil and gently rinsed with tap water, ii) paired with a plant of similar stature, and iii) placed on 3 cm × 6 cm NitroBind pure nitrocellulose paper (0.45 *um*) reinforced with fiberglass mesh and folded into a sandwich secured closed two paperclips (Fig. 1).

Forming hyphal linkages.– Sandwiches were planted into coarse or medium grade sterilized vermiculite at 3-4 sandwiches per pot (1.9 L). To increase survivorship, the sandwiches were placed in a misting room (8 s mist every 8 min) for 5-7 d before returning to the original greenhouse room.

Illuminating direct transfer with Phorwite.− Phorwite BRU Liquid 01 Ê (Bayer Corp.), a membrane impermeant optical brightener, was used as a fluorescent marker molecule to illuminate a direct transfer pathway between linked plants. Phorwite emits bright blue fluorescence (Emission 470 nm) when illuminated by *UV* light (Excitation 370 nm) Phorwite is easily identified in plant tissues. There was negligible non-specific background fluorescence in unexposed plant tissues. Phorwite was not used in subsequent fluorometer studies since there was a relatively high background at 470nm in crushed plant matter and more importantly it failed to produce stable emission readings (quenching) at experimental concentrations.

Phorwite exposure.–Between 6-7 wk of age sandwiches were removed and rinsed of excess vermiculite. The roots of the two IBF plants were independently submerged in 37 ml glass vials containing either donor media, a 1% $^{w/v}$ Phorwite solution or receiver media, a 15% $^{w/v}$ 10,000 MW polyethylene glycol solution (Fig. 2). Polyethylene glycol (PEG), is an osmoticant which produces an osmotic potential of -0.25 ±.03 MPa at 15% $^{w/v}$ (Castonguay, 1992) creating water stress (Kramer and Boyer, 1995). A total of five sandwiches (two IBF sandwich $^{-1}$) were subjected to the donor and receiver medias for 16-18 h (4-5 h light/8 h dark/4-5 h light). A dark or overnight period was deemed necessary since initial screening of light only exposures failed to produce fluorescing hyphae or robust fluorescence in the tips of the lateral roots (unpublished).

Identifying transfer pathway and point of entry sites.– Following the dye exposure the sandwiches were gently opened to minimize hyphal damage and viewed using a Leica FluoCombi III stereomicroscope (8x ï 10x Mag.) with an UV filter. Robust linkage sites (Fig. 3), were identified as sites with three or more hyphal linkages cm^{-1} of receiver root. Root samples (1.5ï 2.0 cm in length) were excised from the receiver root at linkage sites with fluorescing hyphae. The samples were set in 3% agar and micro-sectioned into 150 *u*m thick cross-sections using a Vibratome. The cross-sections were viewed using the stereomicroscope and imaged.

Quantifying net transfer with rhodamine. –Rhodamine G ([RG] excitation 482nm; emission 605nm), a membrane permeant fluorescent dye, produced stable emission readings at 605nm and with relatively low background emission in IBF stems.

Water potential regimes (Rhodamine).− Donor media (DM) and receiver media (RM) were manipulated to create three water regimes treatments: (i) R_{15}, water-stressed receiverï a water potential gradient favoring movement toward the receiver plant, (DM-0.1% RG, RM-15% PEG); (ii) R_{DI}, no water stressï a water gradient with no net movement, (DM-0.1% RG, RM-0% PEG; and (iii) D_{15}, water-stressed donorï a water potential gradient favoring net movement toward the donor plant, (DM-0.1% RG &15% PEG - RM 0% PEG). Two additional fungicide treatments were added R_{15}FUNG R_{DI}FUNG as controls. These sandwiches were treated weekly with dimethyl 4, 4ô-o-phenylenebis (Cleary 3336wpÊ) at a rate of 50mg/100 mL water per pot. Dimethyl 4, 4ô-o-phenylenebis is a systemic fungicide that inhibits arbuscular mycorrhizal development (Carey et al. 2004).

Sandwich Age.– Three age class treatments were tested: 5 d, 6 wk, and 12 wk. Sandwiches aged 5 d and 6 wk were created from IBF plants planted and grown from seed 11/17/06 and the 12 wk old sandwiches were created with IBF plants, planted 10/06/07. Sixteen plants seeded 11/17/06 were harvested 3/13/07 and used to calculate background emission at 605nm for all experimental plants. All donor roots were submerged in 0.1% RG solution and all receiver roots were submerged in a 15% $^{w/v}$ PEG solution to produce a water potential gradient favoring movement toward the receiver plant.

Rhodamine exposure.– The exposure period for water potential and sandwich age treatments consisted of: (i) a 4-5 hour light period, (ii) an 8 hr dark period, and (iii) a 4-6 hour light period. The donor root was placed in the DM between 4:00 and 5:00 pm and removed the following day between 11:00 am and 12:00 pm. The plants were then placed in an oven for at least 48 h at 60 C. For the water potential treatments, the first 12 cm of donor and receiver stems were crushed, weighed, and placed into a 1.5 mL Eppendorf tube. The entire stem was used in the sandwich age treatments. The samples were stored at -4 C. Twenty four hours prior to testing 1.2 mL of deionized water was added to each sample and left overnight at 4 C.

Fluorometer.– Samples were agitated for 2-3 s (Vortex genie2), and placed in a centrifuge (Eppendorf 5417C) at 2000 rpm for 2 min. The supernatant was transferred to a cuvette for reading. The fluorometer (Foci system 3) was calibrated to a baseline intensity reading of zero using de-ionized water and a fullscale reading of 100 using 0.0001% RG $^{w/v}$ solution. The sampling rate was 1 sec^{-1} and the sensitivity at 0.001×. Relative rhodamine concentration was calculated by the gross relative fluorescence units (RFU) at 605nm per sample minus the natural background emission per sample. Non-specific background fluorescence was calculated using sets of plants of the same age class for both experiments.

Calculating net rhodamine transfer.– Percent transfer was calculated as receiver RFU g^{-1} d.w./donor RFU g^{-1} d.w. *100 was used as the experimental variable. Donor stems with 100 or less RFU indicated poor rhodamine uptake and were excluded. Analysis of variance and LSD comparison of mean tests were performed with Statistix 7.1.

RESULTS

The results, generated using phorwite and rhodamine, indicate that direct interplant transfer was facilitated by water potential gradients when two IBF plants were linked. Direct interplant transfer was visually documented using phorwite, a membrane impermeant dye. Rhodamine, a membrane permeable dye, was used to quantify water potential driven molecular transfer.

Illuminating direct transfer with phorwite.ï Phorwite was visible in all the donor stems indicating donor uptake. Receiver root samples were taken from 4 of the 5 sandwiches with visible robust linkage sites. Three samples did not have visible fluorescing hyphae and did not reveal any bright blue fluoresce when micro-sectioned, indicating that if phorwite was present, it was not in high enough concentration to be detected. The root sample associated with visibly fluorescing hyphal linkages (Fig. 3) had bright blue fluorescence when micro-sectioned. Serial sectioning revealed a radial increase in fluorescence and fluorescing structures in six consecutive micro-sections within the root cortex. The structures were consistent with fungal arbuscules. Fluorescence within the stele (Fig. 4) is an indication of direct transfer of the dye from the fungi to the host plant. A fluorescing hyphae remained attached to the root microsection following the fixation and sectioning process (Fig. 5).

Quantifying water potential driven transfer with rhodamine.–The amount of rhodamine transfer was affected by the water relations between the donor and receiver plant (Table. 1). Maximum transfer occurred when the receiver root was water stressed; treatment R15

(Table 1). The average rhodamine concentration in the receiver root was nearly 20 percent of the donor concentration, with an 18 h exposure period. Fungicide treated sandwiches (R15FUNG) had significantly less rhodamine transfer when water stressed. The average rhodamine concentration in the receiver root was 7.4 percent of the donor concentration (Table 1). No significant dye transfer occurred when the water potential gradient favored no net movement (no water stress) or when the donor was water stressed (Table 1). Expected background fluorescence levels of IBF stems at 605 nm were likely overestimated, which resulted in negative percent transfer values (Fig. 6). A negative percent transfer was considered as zero transfer.

Sandwich age experiment.–The amount of rhodamine transfer was affected by the length of time the roots were sandwiched together (Table 2). Six- and twelve-week old sandwiches had significantly more rhodamine transfer than five day-old sandwiches which had no net transfer, when the receiver plant was water stressed (Table 2).

DISCUSSION

Temporal and spatial source/sinks occurring within a common mycorrhizal network (CMN) are believed to facilitate exchange of carbon, nitrogen, and other mineral nutrients (Simard and Durall, 2004). The results of this study indicate that movement of water within a CMN follows the source/sink model. The results suggest that direct interplant solute transfer is facilitated by water potential gradients and that water potential gradients are not arrested at the biotrophic interface. A putative role of water potential facilitated transfer will be discussed regarding: (i) interplant carbon transfer research; (ii) fungal nutrient mineralization; (iii) fungal osmoregulation; (iv) delivery of phytochemical hormones.

A direct transfer pathway

Even though the results of several studies have shown that molecular transfer is enhanced in the presence of AM fungi, the lengthy exposure periods have left open the possibility that the transfer pathway is not direct (Bidartondo et al. 2002; Lerat et al. 2002; Carey et al. 2004). Direct molecular fungal transfer consists of three steps: (i) efflux, molecular transfer from the donor plant into the fungus; (ii), translocation, molecular movement within the fungus, such as across a hyphal bridge; and (ii) influx, molecular transfer from the fungal tissue into the receiver tissue.

Phorwite experiment: evidence of direct transfer

The presence of phorwite in the extraradical hyphal linkages (Fig. 3) indicated the efflux and translocation of the dye into and within the hyphae. Wicking of phorwite along the surface of the fungal was unlikely since, (i) the surface of extraradical hyphal is hydrophobic (Smith and Read, 1997), (ii) there was no evidence of leaching from the hyphae onto either the nitrocellulose paper nor the surfaces of neighboring roots, and (iii) strong fluorescence appears within the receiver root cortex (Fig. 4) not on the root epidermis. These results support a direct fungal point of entry into the receiver plant. The fluorescing structures, within the root cortex (Fig. 4) appeared to be arbuscules. The radial pattern of fluorescence within the root cortex was consistent with a mycorrhizal infection unit first described by Friese and Allen (1991). The brighter fluorescence intensity of the presumed arbuscules suggests they were the site of phorwite efflux into the receiver tissue (Fig. 5). The presence of dye within the receiver root stele (Fig. 4 & 5) is further evidence of dye efflux into the plant tissue, since AM fungal arbuscules are confined to the root cortex (Smith and Read, 1997).

Although only one of five plant pairs was visibly connected by fluorescing hyphae, it does not preclude the occurrence of phorwite transfer between the other plant pairs. To visibly identify a fluorescing hyphal bridge the following conditions must be met: (i) the phorwite concentration must be sufficient to be clearly visible, (ii) the hyphal bridge must remain intact during the opening of the sandwich and (iii) the hyphal bridge must be in plain view.

Rhodamine experiment: evidence of direct water potential facilitated transfer

In this study, when the receiver plant was water-stressed rhodamine (Tables 1 &2) transfer occurred within 18 h. The lack of rhodamine transfer between donor and receiver plant, under no-stress conditions (no water source/sink) discounts diffusion as the primary factor facilitating the interplant dye transfer. The possibility of indirect transfer or root-to-root transfer was reduced since: (i) significantly less rhodamine was transferred between the fungicide treated donor and receiver plants when water-stressed; (ii) there was no evidence of rhodamine transfer in 5-day old sandwiches; and (iii) dye influx into the receiver plant was visualized using phorwite. The results of the study support direct water potential driven transfer of phorwite and rhodamine.

Do water potential gradients facilitate solute transfer?

The transfer of phorwite, a membrane impermeant molecule, as well evidence other membrane impermeant molecules (Querejeta et al. 2003) are transferred through the plant/fungal biotrophic interface by water potential gradients is intriguing. Water potential driven transfer of membrane impermeant molecules through the biotrophic fungi/plant interface suggests that other solutes may be transported in similar fashion.

The carbon transfer debate

The carbon transfer debate is focused on whether direct interplant transfer of carbon occurs. Several studies reported a lack of significant interplant carbon transfer into the receiver plant tissue (Hirrel and Gerdemann 1979; Francis and Read 1984; Martins 1993; Waters and Borowicz 1994; Watkins et al. 1996; Fitter et al. 1998), while other studies reported net interplant carbon transfer (Bidartondo et al. 2002; Lerat et al. 2002; Carey et al. 2004). Resolution of the debate may be achieved through a re-examination of the experimental methods used to facilitate interplant carbon transfer, specifically how carbon source-sink relationship are formed.

Creating opposing carbon and water source/sinks

Carbon transfer studies assume that carbon will move over time, from source (donor plant) to sink (receiver plant). The source is often a donor plant exposed to full light and the sink is a receiver plant that has either been shaded or de-stemmed. Forming carbon source-sinks by this method (Hirrel and Gerdemann 1979; Francis and Read 1984; Fitter et al. 1998) would putatively prevent the formation of a water potential gradient which favors the movement of carbon toward the receiver. Shaded or de-stemmed roots would likely still maintain positive root pressure which creates a water potential gradient favoring movement away from the receiver plant. Determining if water potential gradients affect interplant carbon transfer into the receiver plant would be relatively straightforward using the methods described in this paper.

During water stress does the host provide water to its fungal partner?

The upper soil layers, where AM fungi exist, are prone to water stress. During times of water scarcity having the ability to tap a host for water would obviously benefit the fungi, and if the fungi uses the water to mineralize nutrients destined for the plant, then relationship would in indeed be mutual. Querejeta et al (2003) found that after 70 days of drought, mycorrhizal fungi persisted in soil with water potential values as low as ï 20 MPa. Providing water to the fungi would benefit the plant by ñpriming the pumpò, supplying water to aid the fungi in solublizing mineral nutrients in the soil, destined for the plant. Querejeta et al (2003) found dye in the soil adjacent to the hyphal tips only when dye was injected into soil the previous night which not only provides evidence of water and solute (dyes) influx, but also efflux from the fungi, and most importantly water was delivered to the presumed site of fungal mineralization. More evidence of the host plant functioning as a water source can be found in water relations studies. Using a split-pot format to spatially separate extraradical hyphae from the host plant, extraradical hyphae were reported not to enhance water uptake (George et al, 1997) and even delay soil drying (Auge et al 1994) from the hyphae only compartment, the expected outcome when the hyphae are using water from the host plant.

Fungal Osmoregulation

Aquaporins have been found at the biotrophic interface and plant aquaporins have been shown to acts as channels for known osmolytes, including glycerol (Tyerman, 2002).

The existence of a hydraulic pathway between symbionts makes it reasonable to presume osmosregulatory synergies involving the transfer of water and/or osmolytes across the biotrophic interface during times of water stress.

Are temporal water source sink gradients synchronized?

Diurnal water source sink gradients occurring with a mycorrhizal roots system would produce oscillating water potential gradients which would facilitate water movement towards the host plant during the day (active transpiration) and away from the host toward the fungi at night (no transpiration). If water source sink gradients affect the transfer of soil derived mineral nutrients then the maximum rate of transfer would occur during peak transpiration. Likewise if water potential gradients affect the transfer of plant-derived C to the fungi, then the maximum rate of transfer would follow the cessation of transpiration when root positive pressure is at its maximum. Tyerman et al, (2002) suggested bulk water flow through aquaporins would facilitate the movement of P through the biotrophic towards the host, and impede the movement of sugar from the host plant to the fungi.

Delivery of phytochemicals

It makes evolutionary sense that plants have developed strategies to utilize their fungal symbionts for the direct delivery of phytochemicals. Pinpoint delivery of phytochemicals would be more efficient than broadcasting into rhizosphere. Mycorrhizal inoculation has been shown to significantly increase the levels of several phytohormones in the host plant (Hause et al, 2007). It would be interesting to determine if any of these chemicals find their way into extraradical hyphae, especially during times of water-stress. Assaying extraradical hyphae would be a relatively straightforward process.

Summary

The results of this study indicate water potential gradients facilitate molecular interplant transfer directly through a common mycorrhizal network during water stress. Previous observations of bidirectional movement of tracers within hyphae were interpreted as evidence against water potential gradient driven mass flow (Smith and Read, 1997), since they could not account for movement of tracers away from the host plant. Water potential gradients are essentially a water source/sink, and since plants are believed to diurnally oscillate between negative and positive root pressure (Kramer and Boyer, 1995) then water potential gradients would cause bidirectional movement. Evidence that membrane permeant (rhodamine) and impermeant (phorwite) solutes can be transferred across the biotrophic interface raises the potential that other solutes may be transferred in a similar manner. The same source/sink model applied to the movement of other nutrients within a CMN or a mycorrhizal root should also apply for water.

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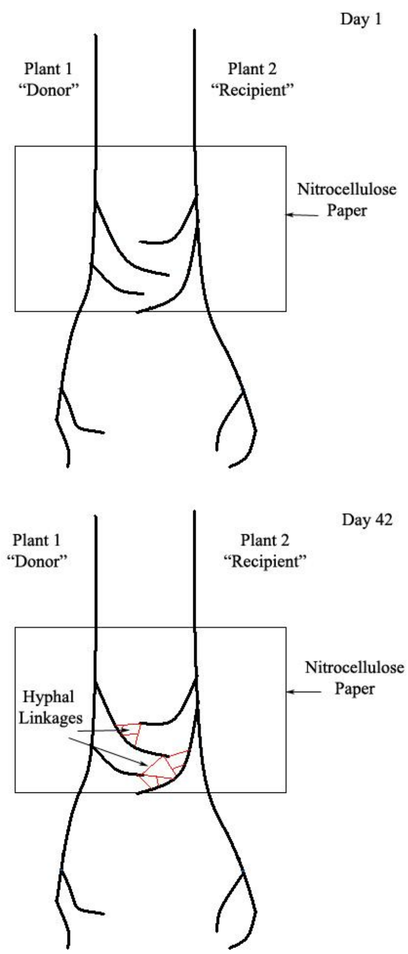

Figure 1**.** Schematic showing the formation of hyphal linkages in nitrocellulose sandwiches at 42 d.

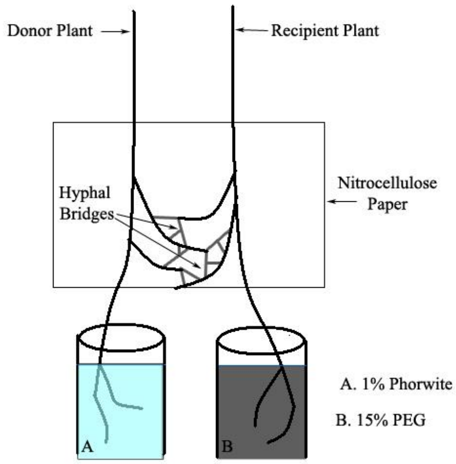

Figure 2**.** Schematic of the dye exposure procedure using the water stressed treatment as an example.

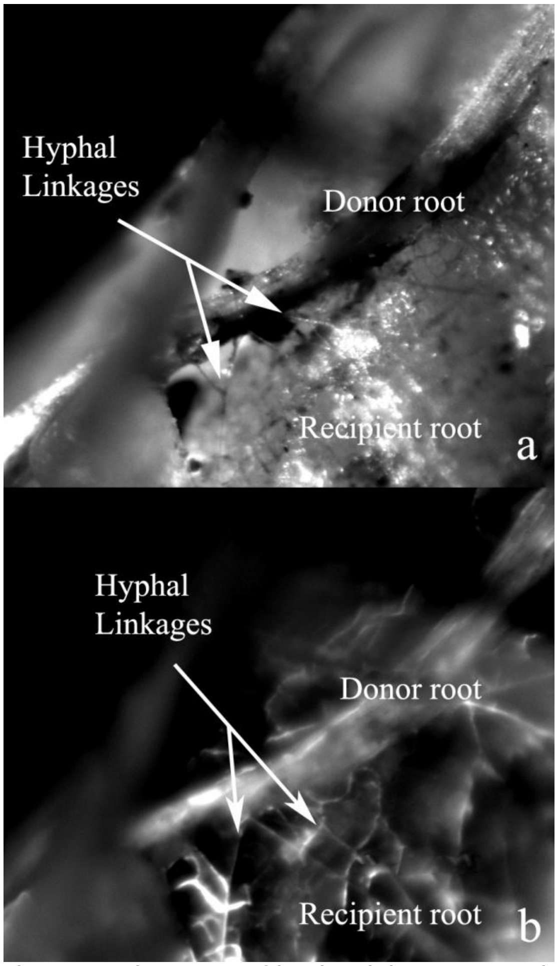

Figure 3**.** Microgram of hyphal linkages occurring between donor and receiver IBF roots. (a) a lateral root of the donor plant linked by hyphae to a receiver root using normal light. (b) with UV light, showing fluorescing hyphal linkages.

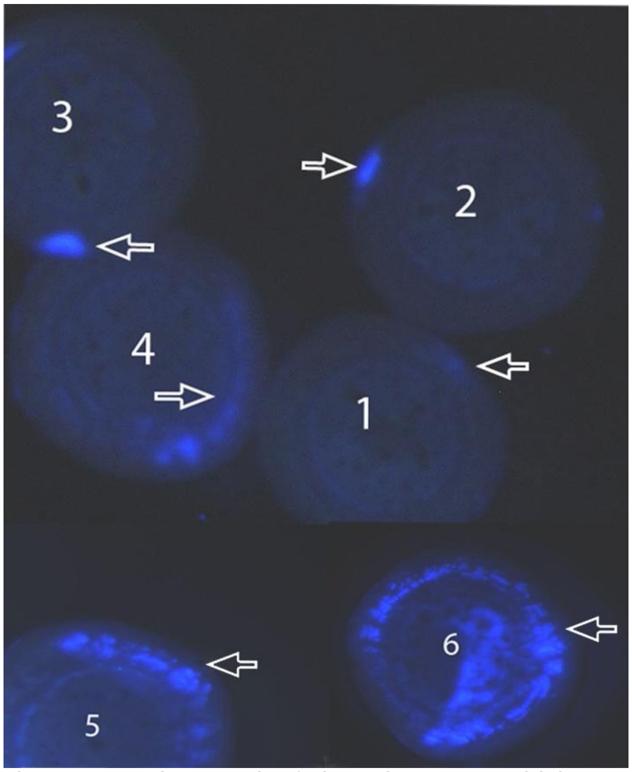

Figure 4. A micrograph of six serial 150 *u*m thick root microsections produced from a receiver IBF root segment with fluorescing hyphal bridges. There is a radial increase of fluorescing structures between sections 1-6.

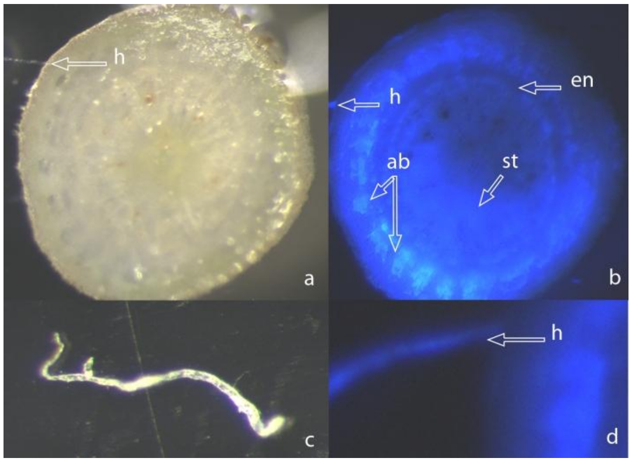

Figure 5. Micrograph of root microsection taken from Fig. 4 (6) of the receiver IBF with attached fluorescing hypha. (a) microsection with attached hyphae (h) observed with normal light. (b) microsection observed with UV light showing the presence of fluorescent dye in both fungal and plant structures and fluorescing hyphae (h), and fungal arbuscules (ab). Dye is also apparent on the interior side of the endodermis (en) within the stele (st), indicating dye transfer from the fungal structures into the plant. (c) detached hyphae under normal light. (d) further magnified image of hyphal attachment with UV light.

Table 1. Comparison of the means LSD test of sandwich age treatment means calculating percent transfer using receiver [RG]/donor [RG]*100.

VARIABLE	MEAN	HOMOGENEOUS GROUPS
R15	19.680	I
R15FNG	7.3789	.. I
RDIFUNG	-1.7154	.. I I
D15	-4.1727	 I
RDI	-14.179	 I

Table 2. Comparison of the means LSD test of sandwich age treatment means calculating percent transfer using receiver [RG]/donor [RG]*100.

VARIABLE	MEAN	HOMOGENEOUS GROUPS
WEEK6	38.575	I
WEEK12	31.600	I
DAY5	-6.5875	.. I

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Appendix A.

Table A.1
Positive pressure: Overnight sap exudatation from IBF roots submerged in distilled water or 15% polyethylene glycol.

Root #	Treatment	Start/stop time	Date	Sap Volume (*u*L)
1	Water	2:00pm/8:00am	12/26/2006	0.9
2	Water	2:00pm/8:00am	12/26/2006	0.4
3	Water	2:00pm/8:00am	12/26/2006	1
4	Water	3:00pm/9:00am	1/5/2007	0.3
5	Water	3:00pm/9:00am	1/5/2007	0.2
6	Water	3:00pm/9:00am	1/5/2007	0.2
7	Water	3:00pm/9:00am	1/5/2007	0.1
8	Water	3:00pm/9:00am	1/5/2007	0.3
9	15%PEG	2:00pm/8:00am	12/26/2006	0
10	15%PEG	2:00pm/8:00am	12/26/2006	0
11	15%PEG	2:00pm/8:00am	12/26/2006	0
12	15%PEG	3:00pm/9:00am	1/5/2007	0
13	15%PEG	3:00pm/9:00am	1/5/2007	0
14	15%PEG	3:00pm/9:00am	1/5/2007	0
15	15%PEG	3:00pm/9:00am	1/5/2007	0
16	15%PEG	3:00pm/9:00am	1/5/2007	0

Table A.2. Transfer of Rhodamine (RG) from a donor plant to a receiver plant using 8 week-old nitrocellulose sandwiches. Treatment R15: Donor 0.1% RG, receiver 15% PEG; no fungicide treatment; overnight.

Sandwich	Donor stem	Receiver stem	Donor gross*	Receiver gross*	Donor BG**	Receiver BG**	Donor net***	Receiver net***	Donor	Receiver	Transfer RG****
(#)	----d.wt (g)----		---------------Relative Fluorescence Units (RFU)----------------						----RFU g^{-1} d.wt----		(%)
1	0.192	0.152	193	150	49.5	47.1	143.5	102.9	747.4	676.8	90.6
2	0.282	0.19	217	125	54.8	49.4	162.2	75.6	575.0	398.0	69.2
3	0.207	0.228	256	70	50.4	51.6	205.6	18.4	993.3	80.5	8.1
4	0.168	0.137	282	156	48.1	46.2	233.9	109.8	1392.4	801.2	57.5
5	0.248	0.213	262	69	52.8	50.7	209.2	18.3	843.5	85.7	10.2
6	0.15	0.123	458	93	47.0	45.4	411.0	47.6	2739.9	386.9	14.1
7	0.163	0.15	347	132	47.8	47.0	299.2	85.0	1835.7	566.6	30.9
8	0.105	0.171	727	61	44.3	48.3	682.7	12.7	6501.5	74.5	1.1
9	0.127	0.124	274	64	45.6	45.5	228.4	18.5	1798.1	149.5	8.3
10	0.184	0.081	411	132	49.0	42.9	362.0	89.1	1967.3	1099.8	55.9
									Mean % receiver [RG]		34.6

*Total fluorescence of stems at 605nm=[rhodamine] + background
**Background fluorescence of stems calculated using y=59.32x+38.11
***Net fluorescence of stems={RG]-background
****% transfer=receiver [RG] g-1 /donor [RG] g-1 *100
¶Fluorometer: fullscale=100, 0.0001%[RG]; baseline=0, H20di;sampling 1 sec; sensitivity x0.001

Table A.3. Transfer of Rhodamine (RG) from a donor plant to a receiver plant using 8 week-old nitrocellulose sandwiches. Treatment R15: Donor 0.1% RG, receiver 15%DI; no fungicide treated.

Sandwich	Donor stem	Receiver stem	Donor gross*	Receiver gross*	Donor BG**	Receiver BG**	Donor net***	Receiver net***	Donor	Receiver	Transfer RG****
(#)	----d.wt (g)----		---------------Relative Fluorescence Units (RFU)---------------						-----RFU g^{-1} d.wt----		(%)
1	0.081	0.037	176	36	42.9	40.3	133.1	-4.3	1643.0	-116.3	-7.1
2	0.075	0.062	À51	34	42.6	41.8	8.4	-7.8	112.5	-125.6	ï
3	0.042	0.067	731	50	40.6	42.1	690.4	7.9	16438.1	118.1	0.7
4	0.133	0.134	1010	117	46.0	46.1	964.0	70.9	7248.1	529.4	7.3
5	0.187	0.124	785	165	49.2	45.5	735.8	119.5	3934.7	964.0	24.5
6	0.045	0.039	724	37	40.8	40.4	683.2	-3.4	15182.7	-87.8	-0.6
7	0.051	0.081	860	120	41.1	42.9	818.9	77.1	16056.2	951.7	5.9
8	0.201	0.188	206	143	50.0	49.3	156.0	93.7	776.0	498.6	64.3
9	0.267	0.212	173	133	53.9	50.7	119.1	82.3	445.9	388.3	87.1
10	0.04	0.083	782	95	40.5	43.0	741.5	52.0	18537.9	626.1	3.4
11	0.024	0.108	1120	96	39.5	44.5	1080.5	51.5	45019.4	476.7	1.1
12	0.11	0.131	548	61	44.6	45.9	503.4	15.1	4576.0	115.4	2.5
13	0.062	0.066	248	72	41.8	42.0	206.2	30.0	3326.0	454.2	13.7
14	0.029	0.037	185	74	39.8	40.3	145.2	33.7	5005.9	910.7	18.2
15	0.021	0.036	890	60	39.4	40.2	850.6	19.8	40506.9	548.7	1.4
16	0.046	0.033	689	60	40.8	40.1	648.2	19.9	14090.5	604.0	4.3
								Mean % receiver [RG]			15.1

ÀPoor donor uptake (RFU<100), data not analyzed
*Total fluorescence of stems at 605nm=[rhodamine] + background
**Background fluorescence of stems calculated using y=59.32x+38.11
***Net fluorescence of stems={RG]-background
****% transfer=receiver [RG] g-1 /donor [RG] g-1 *100
¶Fluorometer: fullscale=100, 0.0001%[RG]; baseline=0, H20di;sampling 1 sec; sensitivity x0.001

Table A.4. Transfer of Rhodamine (RG) from a donor plant to a receiver plant using 8 week-old nitrocellulose sandwiches. Treatment R15: Donor 0.1% RG, receiver 15% PEG; no fungicide; overnight.

Sandwich	Donor stem	Receiver stem	Donor gross*	Receiver gross*	Donor BG**	Receiver BG**	Donor net***	Receiver net***	Donor	Receiver	Transfer RG****
(#)	----d.wt (g)----		----------------Relative Fluorescence Units (RFU)---------------						----RFU g^{-1} d.wt----		(%)
1	0.152	0.175	512	48	47.1	48.5	464.9	-0.5	3058.4	-2.8	-0.1
2	0.125	0.187	442	62	45.5	49.2	396.5	12.8	3171.8	68.4	2.2
3	0.18	0.267	552	72	48.8	53.9	503.2	18.1	2795.6	67.6	2.4
4	0.211	0.278	302	84	50.6	54.6	251.4	29.4	1191.3	105.8	8.9
5	0.259	0.213	445	89	53.5	50.7	391.5	38.3	1511.7	179.6	11.9
6	0.197	0.205	338	64	49.8	50.3	288.2	13.7	1463.0	67.0	4.6
7	0.265	0.298	174	90	53.8	55.8	120.2	34.2	453.5	114.8	25.3
8	0.201	0.207	154	102	50.0	50.4	104.0	51.6	517.2	249.3	48.2
9	0.158	0.217	290	57	47.5	51.0	242.5	6.0	1534.9	27.7	1.8
10	0.072	0.122	148	65	42.4	45.3	105.6	19.7	1466.9	161.1	11.0
							Mean % receiver [RG]				11.6

*Total fluorescence of stems at 605nm=[rhodamine] + background
**Background fluorescence of stems calculated using y=59.32x+38.11
***Net fluorescence of stems={RG}-background
****% transfer=receiver [RG] g-1 /donor [RG] g-1 *100
¶Fluorometer: fullscale=100, 0.0001%[RG]; baseline=0, H20di;sampling 1 sec; sensitivity x0.001

Table A.5. Transfer of Rhodamine (RG) from a donor plant to a receiver plant using 8 week-old nitrocellulose sandwiches. Treatment R15FUNG: Donor 0.1% RG, receiver 15%PEG; fungicide; overnight. SET J 8/30/07

Sandwich	Donor stem	Receiver stem	Donor gross*	Receiver gross*	Donor BG**	Receiver BG**	Donor net***	Receiver net***	Donor	Receiver	Transfer RG****
(#)	----d.wt (g)----		---------------Relative Fluorescence Units (RFU)---------------						----RFU g^{-1} d.wt----		(%)
1	0.152	0.175	512	48	47.1	48.5	464.9	-0.5	3058.4	-2.8	-0.1
2	0.125	0.187	442	32	45.5	49.2	396.5	-17.2	3171.8	-92.0	-2.9
3	0.18	0.267	552	46	48.8	53.9	503.2	-7.9	2795.6	-29.8	-1.1
4	0.211	0.278	302	35	50.6	54.6	251.4	-19.6	1191.3	-70.5	-5.9
5	0.259	0.213	445	89	53.5	50.7	391.5	38.3	1511.7	179.6	11.9
6	0.197	0.205	338	64	49.8	50.3	288.2	13.7	1463.0	67.0	4.6
7	0.265	0.298	174	60	53.8	55.8	120.2	4.2	453.5	14.1	3.1
8	0.201	0.207	154	102	50.0	50.4	104.0	51.6	517.2	249.3	48.2
9	0.158	0.217	290	57	47.5	51.0	242.5	6.0	1534.9	27.7	1.8
10	0.072	0.122	148	65	42.4	45.3	105.6	19.7	1466.9	161.1	11.0
							Mean % receiver [RG]				7.1

*Total fluorescence of stems at 605nm=[rhodamine] + background
**Background fluorescence of stems calculated using y=59.32x+38.11
***Net fluorescence of stems={RG]-background
****% transfer=receiver [RG] g-1 /donor [RG] g-1 *100
¶Fluorometer: fullscale=100, 0.0001%[RG]; baseline=0, H20di;sampling 1 sec; sensitivity x0.001

Table A.6. Transfer of Rhodamine (RG) from a donor plant to a receiver plant using 8 week-old nitrocellulose sandwiches. Treatment R15FUNG: Donor 0.1% RG, receiver 15%PEG; fungicide treated; overnight. SET B 7/9/07

Sandwich	Donor stem	Receiver stem	Donor gross*	Receiver gross*	Donor BG**	Receiver BG**	Donor net***	Receiver net***	Donor	Receiver	Transfer RG****
(#)	----d.wt (g)----		---------------Relative Fluorescence Units (RFU)---------------						----RFU g^{-1} d.wt----		(%)
1	0.138	0.186	304	54	46.3	49.1	257.7	4.9	1867.4	26.1	1.4
2	0.123	0.150	192	79	45.4	47.0	146.6	32.0	1191.8	213.3	17.9
3	0.154	0.180	358	38	47.2	48.8	310.8	-10.8	2017.9	-59.9	-3.0
4	0.098	0.119	164	53	43.9	45.2	120.1	7.8	1225.3	65.8	5.4
5	0.133	0.159	134	76	46.0	47.5	88.0	28.5	661.7	179.0	27.1
6	0.141	0.094	232	57	46.5	43.7	185.5	13.3	1315.8	141.6	10.8
7	0.118	0.101	109	43	45.1	44.1	63.9	-1.1	541.4	-10.9	-2.0
8	0.088	0.096	356	76	43.3	43.8	312.7	32.2	3553.1	335.4	9.4
9	0.089	0.074	435	51	43.4	42.5	391.6	8.5	4400.1	114.9	2.6
								Mean % receiver [RG]			7.7

*Total fluorescence of stems at 605nm=[rhodamine] + background
**Background fluorescence of stems calculated using y=59.32x+38.11
***Net fluorescence of stems={RG]-background
****% transfer=receiver [RG] g-1 /donor [RG] g-1 *100
¶Fluorometer: fullscale=100, 0.0001%[RG]; baseline=0, H20di;sampling 1 sec; sensitivity x0.001

Table A.7. Transfer of Rhodamine (RG) from a donor plant to a receiver plant using 8 week-old nitrocellulose sandwiches. Treatment R15FUNG: Donor 0.1% RG, receiver 15%PEG; fungicide treated; overnight. SET F 7/12/07

Sandwich	Donor stem	Receiver stem	Donor gross*	Receiver gross*	Donor BG**	Receiver BG**	Donor net***	Receiver net***	Donor	Receiver	Transfer RG****
(#)	----d.wt (g)----		---------------Relative Fluorescence Units (RFU)---------------						----RFU g^{-1} d.wt----		(%)
1	0.127	0.191	472	86	45.6	49.4	426.4	36.6	3357.1	191.4	5.7
2	0.155	0.160	312	81	47.3	47.6	264.7	33.4	1707.7	208.7	12.2
3	0.138	0.184	266	83	46.3	49.0	219.7	34.0	1592.1	184.6	11.6
4	0.205	0.167	251	105	50.3	48.0	200.7	57.0	979.2	341.2	34.8
5	0.107	0.131	1196	96	44.5	45.9	1151.5	50.1	10762.	382.6	3.6
6	0.131	0.146	297	70	45.9	46.8	251.1	23.2	1916.9	159.1	8.3
7	0.155	0.138	242	108	47.3	46.3	194.7	61.7	1256.1	447.1	35.6
8	0.142	0.126	603	141	46.5	45.6	556.5	95.4	3918.8	757.3	19.3
								Mean % receiver [RG]			16.4

*Total fluorescence of stems at 605nm=[rhodamine] + background
**Background fluorescence of stems calculated using y=59.32x+38.11
***Net fluorescence of stems={RG]-background
****% transfer=receiver [RG] g-1 /donor [RG] g-1 *100
¶Fluorometer: fullscale=100, 0.0001%[RG]; baseline=0, H20di;sampling 1 sec; sensitivity x0.001

Table A.8. Transfer of Rhodamine (RG) from a donor plant to a receiver plant using 8 week-old nitrocellulose sandwiches. Treatment RDI: Donor 0.1% RG, receiver de-ionized water (DI); no fungicide; overnight. SET M 7/19/07

Sandwich	Donor stem	Receiver stem	Donor gross*	Receiver gross*	Donor BG**	Receiver BG**	Donor net***	Receiver net***	Donor	Receiver	Transfer RG****
(#)	----d.wt (g)----		---------------Relative Fluorescence Units (RFU)---------------						----RFU g^{-1} d.wt----		(%)
1	0.096	0.154	101	24	43.8	47.2	57.2	-23.2	595.8	-150.9	-25.3
2	0.158	0.154	116	23	47.5	47.2	68.5	-24.2	433.7	-157.4	-36.3
3	0.173	0.193	362	36	48.4	49.6	313.6	-13.6	1812.9	-70.3	-3.9
4	0.161	0.193	À52	22	47.7	49.6	4.3	-27.6	27.0	-142.8	ï
5	0.188	0.137	À55	34	49.3	46.2	5.7	-12.2	30.5	-89.3	ï
6	0.109	0.133	251	37	44.6	46.0	206.4	-9.0	1893.8	-67.7	-3.6
7	0.138	0.21	214	36	46.3	50.6	167.7	-14.6	1215.2	-69.4	-5.7
8	0.19	0.169	218	28	49.4	48.1	168.6	-20.1	887.5	-119.1	-13.4
9	0.194	0.118	463	40	49.6	45.1	413.4	-5.1	2130.8	-43.3	-2.0
10	0.163	0.143	137	36	47.8	46.6	89.2	-10.6	547.4	-74.1	-13.5
							Mean % receiver [RG]				-13.0

ÀPoor donor uptake (RFU<100), data not analyzed
*Total fluorescence of stems at 605nm=[rhodamine] + background
**Background fluorescence of stems calculated using y=59.32x+38.11
***Net fluorescence of stems={RG]-background
****% transfer=receiver [RG] g-1 /donor [RG] g-1 *100
¶Fluorometer: fullscale=100, 0.0001%[RG]; baseline=0, H20di;sampling 1 sec; sensitivity x0.001

Table A.9. Transfer of Rhodamine (RG) from a donor plant to a receiver plant using 9 week-old nitrocellulose sandwiches. Treatment RDI: Donor 0.1% RG, receiver de-ionized water (DI); no fungicide treated; overnight. SET K 8/15/07.

Sandwich	Donor stem	Receiver stem	Donor gross*	Receiver gross*	Donor BG**	Receiver BG**	Donor net***	Receiver net***	Donor	Receiver	Transfer RG****
(#)	----d.wt (g)----		---------------Relative Fluorescence Units (RFU)---------------						----RFU g^{-1} d.wt----		(%)
1	0.097	0.16	À68	32	43.9	47.6	24.1	-15.6	248.8	-97.5	ï
2	0.204	0.172	207	56	50.2	48.3	156.8	7.7	768.6	44.7	5.8
3	0.133	0.177	150	15	46.0	48.6	104.0	-33.6	782.0	-189.9	-24.3
4	0.194	0.199	160	28	49.6	49.9	110.4	-21.9	569.0	-110.1	-19.4
5	0.19	0.153	102	31	49.4	47.2	52.6	-16.2	276.9	-105.8	-38.2
6	0.148	0.102	335	31	46.9	44.2	288.1	-13.2	1946.7	-129.0	-6.6
7	0.108	0.072	À84	26	44.5	42.4	39.5	-16.4	365.6	-227.5	ï
8	0.197	0.156	221	31	49.8	47.4	171.2	-16.4	869.1	-104.9	-12.1
							Mean % receiver [RG]				-15.8

ÀPoor donor uptake (RFU<100), data not analyzed
*Total fluorescence of stems at 605nm=[rhodamine] + background
**Background fluorescence of stems calculated using y=59.32x+38.11
***Net fluorescence of stems={RG]-background
****% transfer=receiver [RG] g-1 /donor [RG] g-1 *100
¶Fluorometer: fullscale=100, 0.0001%[RG]; baseline=0, H20di;sampling 1 sec; sensitivity x0.001

Table A.10. Transfer of Rhodamine (RG) from a donor plant to a receiver plant using 7 week-old nitrocellulose sandwiches. Treatment RDIFUNG: Donor 0.1% RG, receiver de-ionized water (DI); fungicide treated; overnight. SET E 7/12/09

Sandwich	Donor stem	Receiver stem	Donor gross*	Receiver gross*	Donor BG**	Receiver BG**	Donor net***	Receiver net***	Donor	Receiver	Transfer RG****
(#)	----d.wt (g)----		---------------Relative Fluorescence Units (RFU)---------------						----RFU g^{-1} d.wt----		(%)
1	0.113	0.188	À51	51	44.8	49.3	6.2	1.7	54.8	9.2	ï
2	0.121	0.151	158	47	45.3	47.1	112.7	-0.1	931.5	-0.4	0.0
3	0.134	0.116	À88	53	46.1	45.0	41.9	8.0	313.0	69.0	ï
4	0.161	0.122	À96	60	47.7	45.3	48.3	14.7	300.2	120.1	ï
5	0.116	0.093	196	47	45.0	43.6	151.0	3.4	1301.8	36.3	2.8
6	0.139	0.158	108	43	46.4	47.5	61.6	-4.5	443.5	-28.4	-6.4
7	0.18	0.111	À90	52	48.8	44.7	41.2	7.3	229.0	65.8	ï
8	0.131	0.111	110	49	45.9	44.7	64.1	4.3	489.5	38.8	7.9
9	0.062	0.082	192	42	41.8	43.0	150.2	-1.0	2422.8	-11.9	-0.5
10	0.146	0.095	130	44	46.8	43.7	83.2	0.3	570.1	2.7	0.5
							Mean % receiver [RG]				0.7

ÀPoor donor uptake (RFU<100), data not analyzed
*Total fluorescence of stems at 605nm=[rhodamine] + background
**Background fluorescence of stems calculated using y=59.32x+38.11
***Net fluorescence of stems={RG}-background
****% transfer=receiver [RG] g-1 /donor [RG] g-1 *100
¶Fluorometer: fullscale=100, 0.0001%[RG]; baseline=0, H20di;sampling 1 sec; sensitivity x0.001

Table A.11. Transfer of Rhodamine (RG) from a donor plant to a receiver plant using 8 week-old nitrocellulose sandwiches. Treatment RDIFUNG: Donor 0.1% RG, receiver de-ionized water (DI); fungicide treated; overnight. SET D 7/16/07

Sandwich	Donor stem	Receiver stem	Donor gross*	Receiver gross*	Donor BG**	Receiver BG**	Donor net***	Receiver net***	Donor	Receiver	Transfer RG****
(#)	----d.wt (g)----		---------------Relative Fluorescence Units (RFU)---------------						----RFU g^{-1} d.wt----		(%)
1	0.114	0.116	222	38	44.9	45.0	177.1	-7.0	1553.8	-60.3	-3.9
2	0.156	0.102	509	36	47.4	44.2	461.6	-8.2	2959.2	-80.0	-2.7
3	0.151	0.15	540	48	47.1	47.0	492.9	1.0	3264.5	6.6	0.2
4	0.093	0.125	379	47	43.6	45.5	335.4	1.5	3606.2	11.8	0.3
5	0.246	0.209	164	36	52.7	50.5	111.3	-14.5	452.4	-69.4	-15.3
6	0.125	0.124	353	32	45.5	45.5	307.5	-13.5	2459.8	-108.6	-4.4
7	0.077	0.085	632	38	42.7	43.2	589.3	-5.2	7653.5	-60.6	-0.8
							Mean % receiver [RG]				-3.8

ÀPoor donor uptake (RFU<100), data not analyzed
*Total fluorescence of stems at 605nm=[rhodamine] + background
**Background fluorescence of stems calculated using y=59.32x+38.11
***Net fluorescence of stems={RG]-background
****% transfer=receiver [RG] g-1 /donor [RG] g-1 *100
¶Fluorometer: fullscale=100, 0.0001%[RG]; baseline=0, H20di;sampling 1 sec; sensitivity x0.001

Table A.12. Transfer of Rhodamine (RG) from a donor plant to a receiver plant using 8 week-old nitrocellulose sandwiches. Treatment D15: Donor 0.1% RG, PEG %15; receiver de-ionized water; no fungicide; overnight. Date: 7/19/07 SET C

Sandwich	Donor stem	Receiver stem	Donor gross*	Receiver gross*	Donor BG**	Receiver BG**	Donor net***	Receiver net***	Donor	Receiver	Transfer RG****
(#)	----d.wt (g)----		---------------Relative Fluorescence Units (RFU)---------------						----RFU g^{-1} d.wt----		(%)
1	0.188	0.204	649	63	49.3	50.2	599.7	12.8	3190.1	62.7	2.0
2	0.122	0.08	115	30	45.3	42.9	69.7	-12.9	570.9	-160.7	-28.1
3	0.108	0.136	152	47	44.5	46.2	107.5	0.8	995.2	6.0	0.6
4	0.154	0.162	1224	56	47.2	47.7	1176.8	8.3	7641.3	51.1	0.7
5	0.096	0.077	268	50	43.8	42.7	224.2	7.3	2335.4	95.1	4.1
6	0.165	0.127	324	52	47.9	45.6	276.1	6.4	1673.3	50.1	3.0
7	0.193	0.144	À65	37	49.6	46.7	15.4	-9.7	80.0	-67.0	ï
8	0.124	0.151	À86	41	45.5	47.1	40.5	-6.1	326.9	-40.2	ï
9	0.143	0.141	À92	49	46.6	46.5	45.4	2.5	317.5	17.9	ï
10	0.101	0.102	182	34	44.1	44.2	137.9	-10.2	1365.3	-99.6	-7.3
							Mean % receiver [RG]				-3.6

ÀPoor donor uptake (RFU<100), data not analyzed
*Total fluorescence of stems at 605nm=[rhodamine] + background
**Background fluorescence of stems calculated using y=59.32x+38.11
***Net fluorescence of stems={RG}-background
****% transfer=receiver [RG] g-1 /donor [RG] g-1 *100
¶Fluorometer: fullscale=100, 0.0001%[RG]; baseline=0, H20di;sampling 1 sec; sensitivity x0.001

Table A.13. Transfer of Rhodamine (RG) from a donor plant to a receiver plant using 8 week-old nitrocellulose sandwiches. Treatment D15: Donor 0.1% RG, PEG %15; receiver de-ionized water; no fungicide; overnight. Date: 7/17/07 SET I

Sandwich	Donor stem	Receiver stem	Donor gross*	Receiver gross*	Donor BG**	Receiver BG**	Donor net***	Receiver net***	Donor	Receiver	Transfer RG****
(#)	----d.wt (g)----		---------------Relative Fluorescence Units (RFU)---------------						----RFU g^{-1} d.wt----		(%)
1	0.173	0.25	À64	26	48.4	52.9	15.6	-26.9	90.3	-107.8	ï
2	0.133	0.18	À82	49	46.0	48.8	36.0	0.2	270.7	1.2	ï
3	0.18	0.231	101	36	48.8	51.8	52.2	-15.8	290.1	-68.5	-23.6
4	0.075	0.073	240	44	42.6	42.4	197.4	1.6	2632.5	21.4	0.8
5	0.153	0.167	202	22	47.2	48.0	154.8	-26.0	1011.9	-155.8	-15.4
6	0.172	0.143	À89	36	48.3	46.6	40.7	-10.6	236.6	-74.1	ï
7	0.119	0.153	127	42	45.2	47.2	81.8	-5.2	687.7	-33.9	-4.9
8	0.14	0.131	À86	40	46.4	45.9	39.6	-5.9	282.8	-44.9	ï
9	0.132	0.2	152	31	45.9	50.0	106.1	-19.0	803.5	-94.9	-11.8
							Mean % receiver [RG]				-11.0

ÀPoor donor uptake (RFU<100), data not analyzed
*Total fluorescence of stems at 605nm=[rhodamine] + background
**Background fluorescence of stems calculated using y=59.32x+38.11
***Net fluorescence of stems={RG]-background
****% transfer=receiver [RG] g-1 /donor [RG] g-1 *100
¶Fluorometer: fullscale=100, 0.0001%[RG]; baseline=0, H20di;sampling 1 sec; sensitivity x0.001

Table A.14. Transfer of Rhodamine (RG) from a donor plant to a receiver plant using 8 week-old nitrocellulose sandwiches. Treatment D15: Donor 0.1% RG, PEG %15; receiver de-ionized water; no fungicide; overnight. Date: 7/19/07

Sandwich	Donor stem	Receiver stem	Donor gross*	Receiver gross*	Donor BG**	Receiver BG**	Donor net***	Receiver net***	Donor	Receiver	Transfer RG****
(#)	----d.wt (g)----		---------------Relative Fluorescence Units (RFU)---------------						----RFU g^{-1} d.wt----		(%)
1	0.174	0.204	342	39	48.4	50.2	293.6	-11.2	1687.2	-55.0	-3.3
2	0.194	0.206	390	46	49.6	50.3	340.4	-4.3	1754.5	-21.0	-1.2
3	0.147	0.118	384	38	46.8	45.1	337.2	-7.1	2293.7	-60.3	-2.6
4	0.128	0.219	512	42	45.7	51.1	466.3	-9.1	3642.9	-41.6	-1.1
5	0.131	0.105	353	49	45.9	44.3	307.1	4.7	2344.4	44.4	1.9
6	0.144	0.162	594	48	46.7	47.7	547.3	0.3	3801.0	1.7	0.0
7	0.139	0.096	118	43	46.4	43.8	71.6	-0.8	515.4	-8.4	-1.6
8	0.147	0.148	268	46	46.8	46.9	221.2	-0.9	1504.6	-6.0	-0.4
9	0.126	0.119	314	42	45.6	45.2	268.4	-3.2	2130.3	-26.6	-1.3
10	0.154	0.103	445	38	47.2	44.2	397.8	-6.2	2582.8	-60.4	-2.3
							Mean % receiver [RG]				-1.2

*Total fluorescence of stems at 605nm=[rhodamine] + background
**Background fluorescence of stems calculated using y=53.32x+38.11
***Net fluorescence of stems={RG}-background
****% transfer=receiver [RG] g-1 /donor [RG] g-1 *100
Fluorometer: fullscale=100, 0.0001%[RG]; baseline=0, H20di;sampling 1 sec; sensitivity x0.001

Table A.15. Transfer of Rhodamine (RG) from a donor plant to a receiver plant using 12 week-old nitrocellulose sandwiches. Treatment 12WeekR15: Donor 0.1% RG; receiver PEG %15; no fungicide; overnight. Planted 10/06/06; Sandwich 12/16/06; Test 3/13/07

Sandwich	Donor stem	Receiver stem	Donor gross*	Receiver gross*	Donor BG**	Receiver BG**	Donor net***	Receiver net***	Donor [RG]	Receiver [RG]	Transfer RG****
(#)	----d.wt (g)----		---------------Relative Fluorescence Units (RFU)---------------						----RFU g^{-1} d.wt----		(%)
1	0.182	0.118	301	132	68.2	52.6	232.8	79.4	1279.2	673.2	52.6
2	0.195	0.156	539	132	71.4	61.8	467.6	70.2	2398.2	449.7	18.8
3	0.16	0.187	372	92	62.8	69.4	309.2	22.6	1932.4	120.8	6.3
4	0.175	0.174	100	92	66.5	66.2	33.5	25.8	191.6	148.1	ï
5	0.194	0.196	324	110	71.1	71.6	252.9	38.4	1303.5	195.9	15.0
6	0.263	0.189	270	184	88.0	69.9	182.0	114.1	692.2	603.7	87.2
7	0.219	0.159	220	95	77.2	62.6	142.8	32.4	652.0	204.0	31.3
8	0.162	0.248	558	160	63.3	84.3	494.7	75.7	3053.7	305.3	10.0
							Mean % receiver [RG]				31.6

*Total fluorescence of stems at 605nm=[rhodamine] + background
**Background fluorescence of stems calculated using y=244.1x+23.76
***Net fluorescence of stems={RG]-background
****% transfer=receiver [RG] g-1 /donor [RG] g-1 *100
Fluorometer: fullscale=100, 0.0001%[RG]; baseline=0, H20di;sampling 1 sec; sensitivity x0.001

Table A.16. Transfer of Rhodamine (RG) from a donor plant to a receiver plant using 6 week-old nitrocellulose sandwiches. Treatment 6WeekR15: Donor 0.1% RG, receiver PEG %15; no fungicide; overnight. Planted 11/17/06; Sandwich 1/31//07; Test 3/13/07

Sandwich	Donor stem	Receiver stem	Donor gross*	Receiver gross*	Donor BG**	Receiver BG**	Donor net***	Receiver net***	Donor [RG]	Receiver [RG]	Transfer RG****
(#)	----d.wt (g)----		---------------Relative Fluorescence Units (RFU)---------------						----RFU g^{-1} d.wt----		(%)
1	0.08	0.125	131	90	43.3	54.3	87.7	35.7	1096.4	285.8	26.1
2	0.151	0.132	230	81	60.6	56.0	169.4	25.0	1121.7	189.5	16.9
3	0.064	0.114	202	106	39.4	51.6	162.6	54.4	2540.9	477.3	18.8
4	0.175	0.124	160	105	66.5	54.0	93.5	51.0	534.4	411.1	76.9
5	0.186	0.111	172	122	69.2	50.9	102.8	71.1	552.9	640.9	115.9
6	0.097	0.115	192	72	47.4	51.8	144.6	20.2	1490.3	175.4	11.8
7	0.17	0.192	306	68	65.3	70.6	240.7	-2.6	1416.1	-13.7	-1.0
8	0.173	0.192	120	82	66.0	70.6	54.0	11.4	312.2	59.2	19.0
							Mean % receiver [RG]				35.5

*Total fluorescence of stems at 605nm=[rhodamine] + background
**Background fluorescence of stems calculated using y=244.1x+23.76
***Net fluorescence of stems={RG}-background
****% transfer=receiver [RG] g-1 /donor [RG] g-1 *100
Fluorometer: fullscale=100, 0.0001%[RG]; baseline=0, H20di;sampling 1 sec; sensitivity x0.001

Table A.17. Transfer of Rhodamine (RG) from a donor plant to a receiver plant using 6 week-old nitrocellulose sandwiches. Treatment 6WeekR15: Donor 0.1% RG, receiver PEG %15; no fungicide; overnight. Planted 11/17/06; Sandwich 1/31/07; Test 3/14/07

Sandwich	Donor stem	Receiver stem	Donor gross*	Receiver gross*	Donor BG**	Receiver BG**	Donor net***	Receiver net***	Donor [RG]	Receiver [RG]	Transfer RG****
(#)	----d.wt (g)----		---------------Relative Fluorescence Units (RFU)---------------						----RFU g^{-1} d.wt----		(%)
1	0.189	0.165	225	80	69.9	64.0	155.1	16.0	820.7	96.7	11.8
2	0.209	0.158	118	81	74.8	62.3	43.2	18.7	206.8	118.2	57.1
3	0.189	0.191	167	97	69.9	70.4	97.1	26.6	513.8	139.4	27.1
4	0.19	0.188	191	115	70.1	69.7	120.9	45.3	636.1	241.2	37.9
5	0.187	0.179	188	93	69.4	67.5	118.6	25.5	634.2	142.7	22.5
6	0.181	0.151	233	115	67.9	60.6	165.1	54.4	911.9	360.1	39.5
7	0.176	0.145	137	116	66.7	59.2	70.3	56.8	399.3	392.0	98.2
8	0.165	0.173	138	96	64.0	66.0	74.0	30.0	448.3	173.5	38.7
							Mean % receiver [RG]				41.6

*Total fluorescence of stems at 605nm=[rhodamine] + background
**Background fluorescence of stems calculated using y=244.1x+23.76
***Net fluorescence of stems={RG]-background
****% transfer=receiver [RG] g-1 /donor [RG] g-1 *100
Fluorometer: fullscale=100, 0.0001%[RG]; baseline=0, H20di;sampling 1 sec; sensitivity x0.001

Table A.18. Transfer of Rhodamine (RG) from a donor plant to a receiver plant using 5-day old nitrocellulose sandwiches. Treatment 5DayR15: Donor 0.1% RG; receiver PEG %15; no fungicide; overnight. Planted 11/17/06; Sandwich 3/9/07; Test 3/14/07

Sandwich	Donor stem	Receiver stem	Donor gross*	Receiver gross*	Donor BG**	Receiver BG**	Donor net***	Receiver net***	Donor [RG]	Receiver [RG]	Transfer RG****
(#)	----d.wt (g)----		---------------Relative Fluorescence Units (RFU)---------------						----RFU g^{-1} d.wt----		(%)
1	0.089	0.11	129	46	45.5	50.6	83.5	-4.6	938.4	-41.9	-4.5
2	0.11	0.082	172	39	50.6	43.8	121.4	-4.8	1103.5	-58.2	-5.3
3	0.149	0.099	130	44	60.1	47.9	69.9	-3.9	468.9	-39.7	-8.5
4	0.2	0.175	123	60	72.6	66.5	50.4	-6.5	252.1	-37.0	-14.7
5	0.123	0.085	180	46	53.8	44.5	126.2	1.5	1026.1	17.5	1.7
6	0.073	0.047	240	42	41.6	35.2	198.4	6.8	2718.1	144.0	5.3
7	0.126	0.146	147	62	54.5	59.4	92.5	2.6	734.0	17.8	2.4
8	0.173	0.19	104	58	66.0	70.1	38.0	-12.1	219.7	-63.9	-29.1
								Mean % receiver [RG]			-5.3

*Total fluorescence of stems at 605nm=[rhodamine] + background
**Background fluorescence of stems calculated using y=244.1x+23.76
***Net fluorescence of stems={RG]-background
****% transfer=receiver [RG] g-1 /donor [RG] g-1 *100
Fluorometer: fullscale=100, 0.0001%[RG]; baseline=0, H20di;sampling 1 sec; sensitivity x0.001

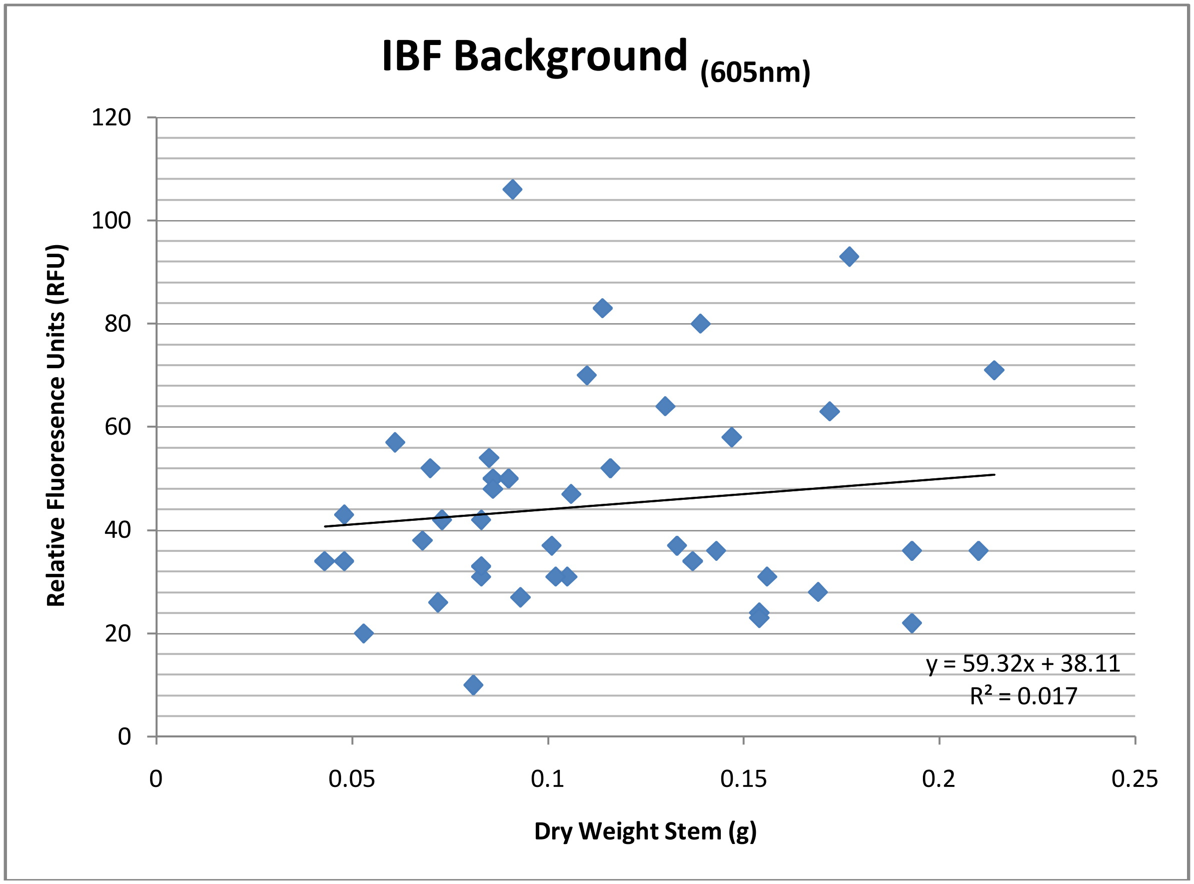

Figure A.1. Background fluorescence of Illinois Bundle Flower stems at 605 nm. Slope = 59.32x + 38.11 used to calculate background for experimental plants. Plot generated using pooled data from two separate age-class plants.

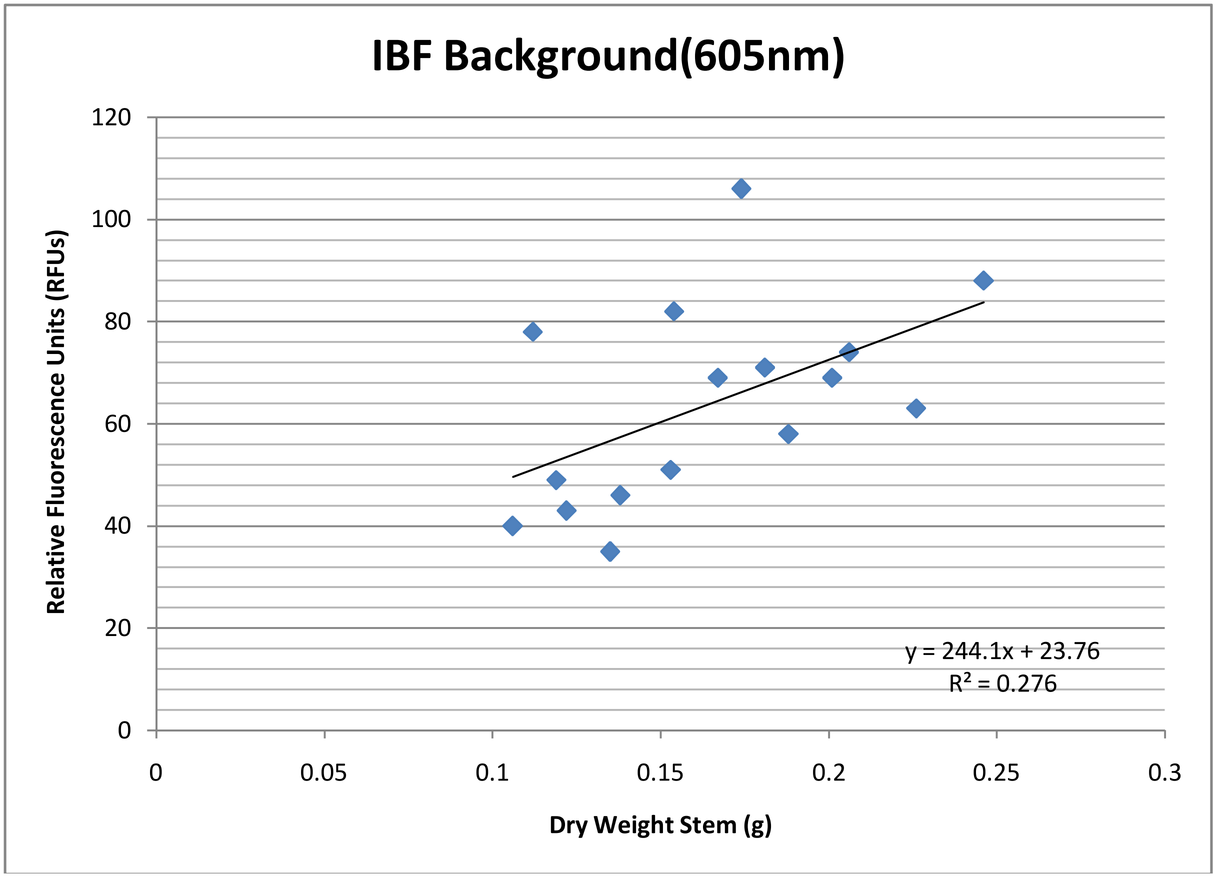

Figure A.2. Background fluorescence of Illinois Bundle Flower stems at 605 nm. Slope = 244.1x + 23.76 used to calculate background for experimental plants (Sandwich Age). Plot generated using data from two separate age-class plants, planted 10/6/06 and 11/17/06.

Table A.19. Analysis of variance examining the effects of water potential regimes and fungicide treatments on rhodamine transfer to receiver stems. Rhodamine concentration calculated using relative fluorescence units per 12 cm of stem.

SOURCE	DF	SS	MS	F	P
Pressure trt	4	50442.8	12610.7	23.63	<0.0001
Error	98	52293.7	533.609		

Table A.20. Water potential treatment means and standard deviations calculated using relative fluorescence units per 12 cm of receiver stem.

VARIABLE	MEAN	SIZE	STD DEV
R15	41.911	35	35.171
R15FUNG	10.716	19	19.213
RDI	-15.286	14	9.7969
RDIFUNG	-3.3462	13	6.0887
D15	-4.0773	22	9.5070
TOTAL	12.848	103	23.100

Table A.21. Water potential comparison of means LSD test of relative fluorescence units per 12 cm of receiver stem.

VARIABLE	MEAN	HOMOGENEOUS GROUPS
R15	41.911	I
R15FUNG	10.716	.. I
RDIFUNG	-3.3462	.. I I
D15	-4.0773	 I
RDI	-15.286	 I

*There are 3 groups in which the means are not significantly different from one another.
**Critical T value 1.984; Rejection level 0.050

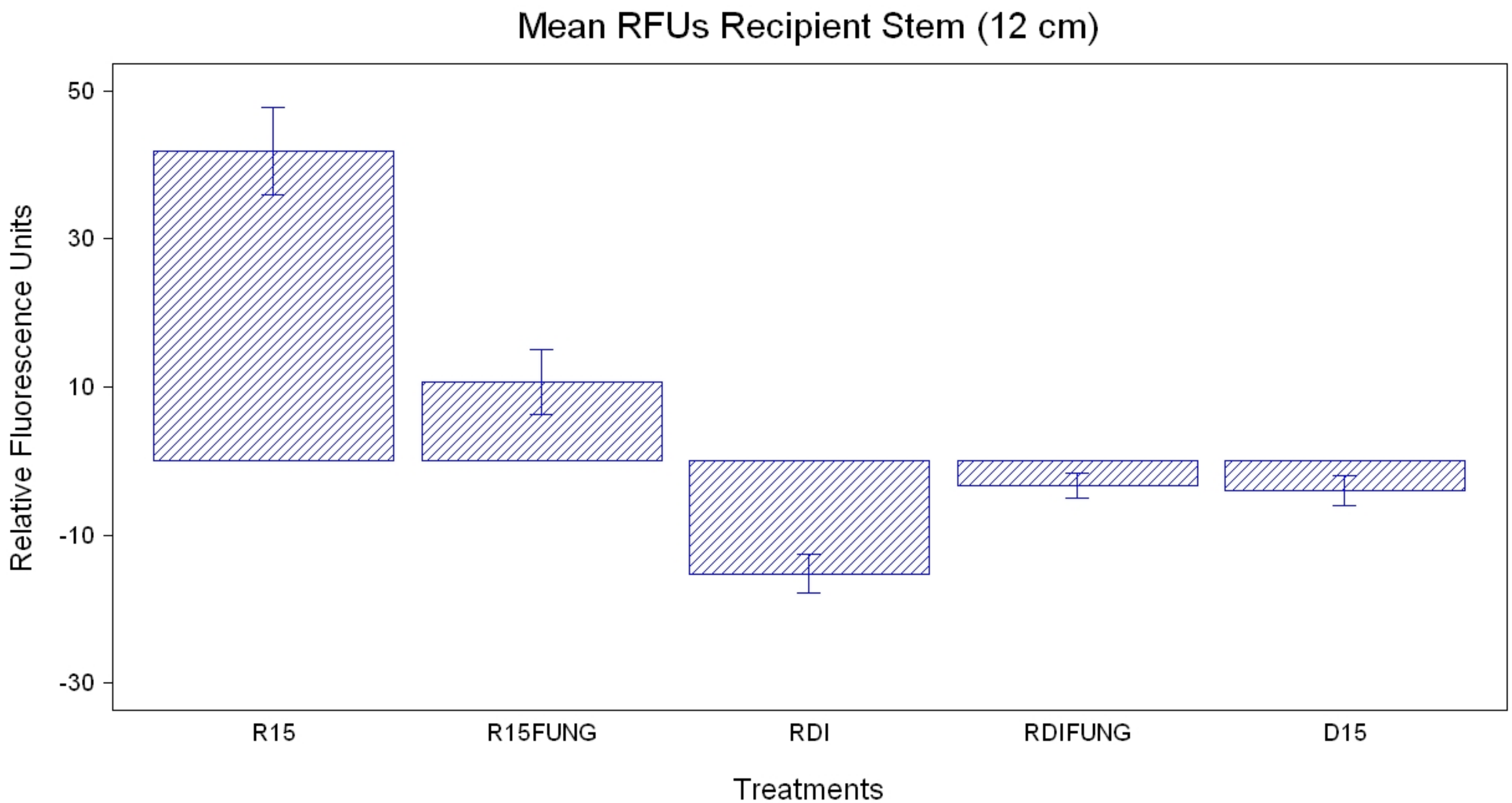

Figure A.3. Mean relative fluorescence units $_{(605nm)}$ per 12 cm of receiver (*Desmanthus illinioensis*) stem with ± SE for treatments. Treatment abbreviations: R receiver media, D donor media, 15 fifteen percent polyethylene glycol, FUNG fungicide treated sandwiches. Statistically significant were detected between treatments by analysis of variance and LSD comparison of the means test.

Table A.22. Analysis of variance examining the effects of water potential regimes and fungicide treatments on rhodamine transfer to receiver stems. Rhodamine concentration calculated using relative fluorescence units per gram d.w. stem.

SOURCE	DF	SS	MS	F	P
Treatments	4	3311921	827980	19.84	<0.0001
Error	98	4088841	41722.9		

Table A.23. Water potential treatment means and standard deviations calculated as relative fluorescence units per g d.w. of stem.

VARIABLE	MEAN	SIZE	STD DEV
R15	352.57	35	328.68
R15FUNG	79.42	19	118.39
RDI	-96.229	14	57.392
RDIFUNG	-24.877	13	46.757
D15	-25.464	22	67.068
TOTAL	112.80	103	204.54

Table A.24. Comparison of the means LSD test of water potential treatment means using relative fluorescence units per g d.w. receiver stem.

VARIABLE	MEAN	HOMOGENEOUS GROUPS
R15	352.57	I
R15FUNG	79.421	.. I
RDIFUNG	-24.877	.. I I
D15	-25.464	.. I I
RDI	-96.229	 I

*There are 3 groups in which the means are not significantly different from one another.
**Critical T value 1.984; rejection level 0.050

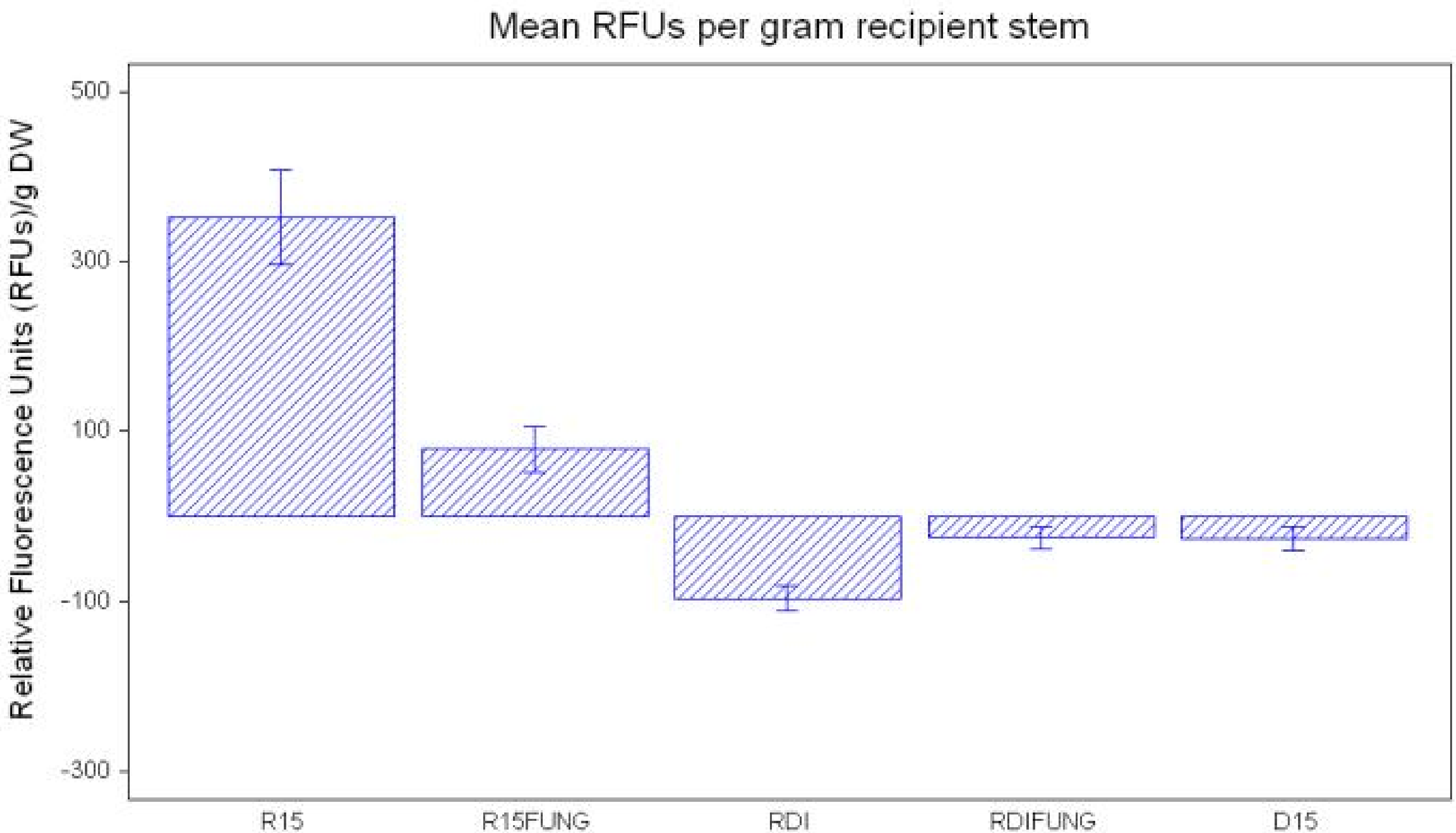

Figure A.4. Mean relative fluorescence units $_{(605nm)}$ per gram d.w. of receiver (*Desmanthus illinioensis*) stem with ± SE for treatments. Treatment abbreviations: R receiver media, D donor media, 15 fifteen percent polyethylene glycol, FUNG fungicide treated sandwiches. Statistically significant were detected between treatments by analysis of variance and LSD comparison of the means test.

Table A.25. Analysis of variance examining the effects of water potential regimes with or without fungicide on rhodamine transfer from donor plants to receiver stems. Percent receiver stem rhodamine [RG] concentration calculated by receiver [RG]/donor [RG]*100.

SOURCE	DF	SS	MS	F	P
Treatments	4	15236.8	3809.21	12.19	<0.0001
Error	98	30617.2	312.420		
TOTAL	102	45854.0			

Table A.26. Water potential treatment means and standard deviations for percent transfer calculated by receiver [RG]/donor [RG]*100.

VARIABLE	MEAN	SIZE	STD DEV
R15	19.680	35	26.360
R15FUNG	7.3786	19	12.781
RDI	-14.179	14	13.066
RDIFUNG	-1.7154	13	5.4020
D15	-4.1727	22	8.4010
TOTAL	5.0136	103	17.675

Table A.27. Comparison of the means LSD test of sandwich age treatment means calculating percent transfer using receiver [RG]/donor [RG]*100.

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VARIABLE	MEAN	HOMOGENEOUS GROUPS
R15	19.680	I
R15FUNG	7.3789	.. I
RDIFUNG	-1.7154	.. I I
D15	-4.1727	 I
RDI	-14.179	 I

*There are 3 groups in which the means are not significantly different from one another.

**Critical T value 1.984; rejection level 0.050

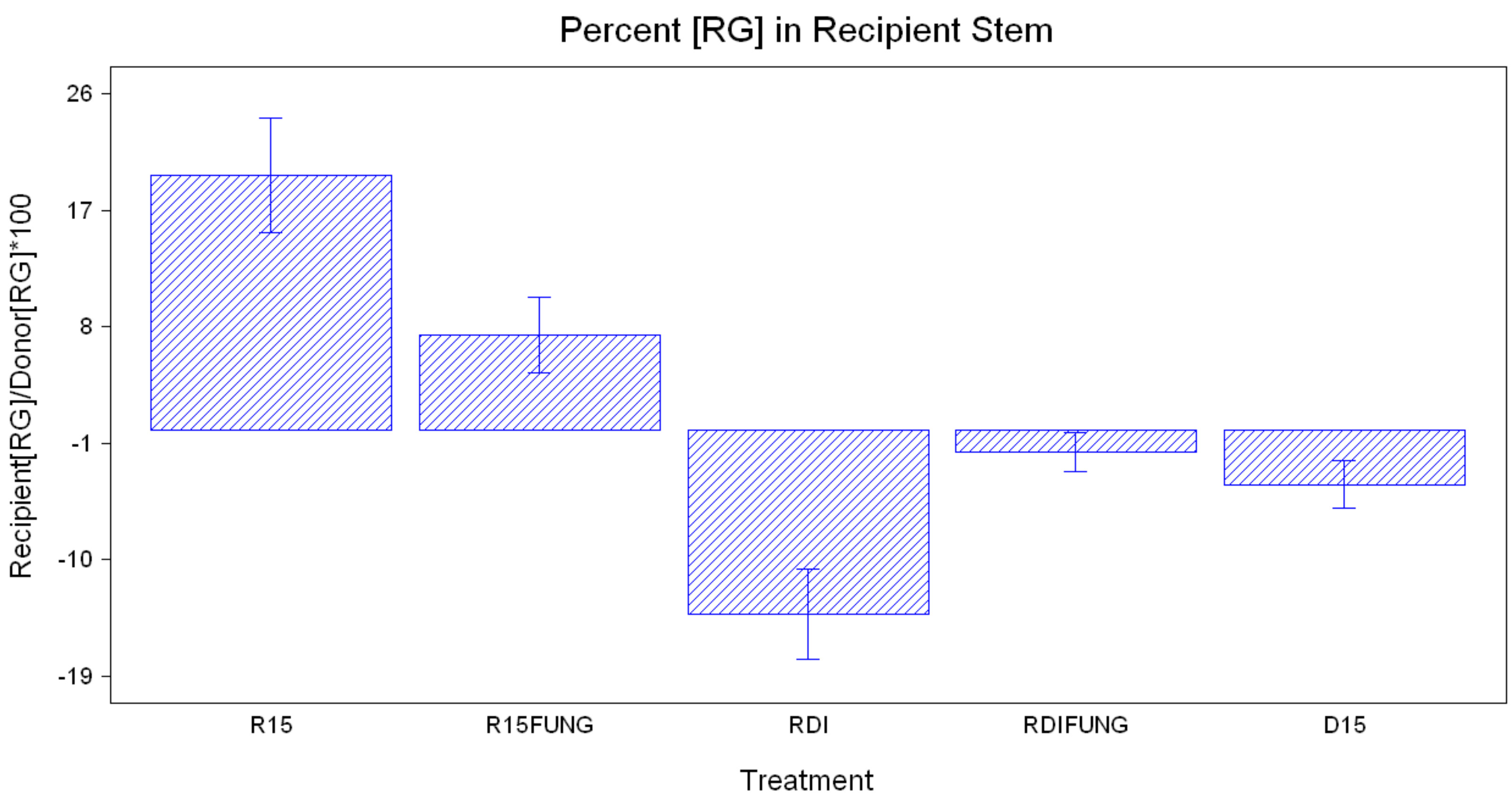

Figure A.5. Percent relative fluorescence units (RFU) per gram d.w. in receiver (*Desmanthus illinioensis*) stem versus with RFUs per gram in donor stems with ± SE. Treatment abbreviations: R receiver media, D donor media, 15 fifteen percent polyethylene glycol, FUNG fungicide treated sandwiches. Statistically significance was detected between treatments by analysis of variance and LSD comparison of the means test.

Table A.28. Analysis of variance examining the effects of sandwich age on rhodamine transfer to receiver stems. Rhodamine concentration calculated using relative fluorescence units (RFU) per 12 cm of stem.

SOURCE	DF	SS	MS	F	P
Treatments	2	563460	281730	10.40	0.0004
Error	28	758849	27101.7		

Table A.29. Sandwich age treatment means and standard deviations calculated using relative fluorescence units per 12 cm of receiver stem.

VARIABLE	MEAN	SIZE	STD DEV
DAY5	-7.6750	8	68.722
WEEK6	243.08	16	173.00
WEEK12	364.66	7	214.81
TOTAL	205.82	31	164.63

Table A.30. Comparison of the means LSD test of sandwich age treatment means using relative fluorescence units per 12 cm receiver stem.

VARIABLE	MEAN	HOMOGENEOUS GROUPS
WEEK12	364.66	I
WEEK6	243.08	I
DAY5	-7.6750	.. I

*There are 2 groups in which the means are not significantly different from one another.
**Critical T value 2.084; rejection level 0.050

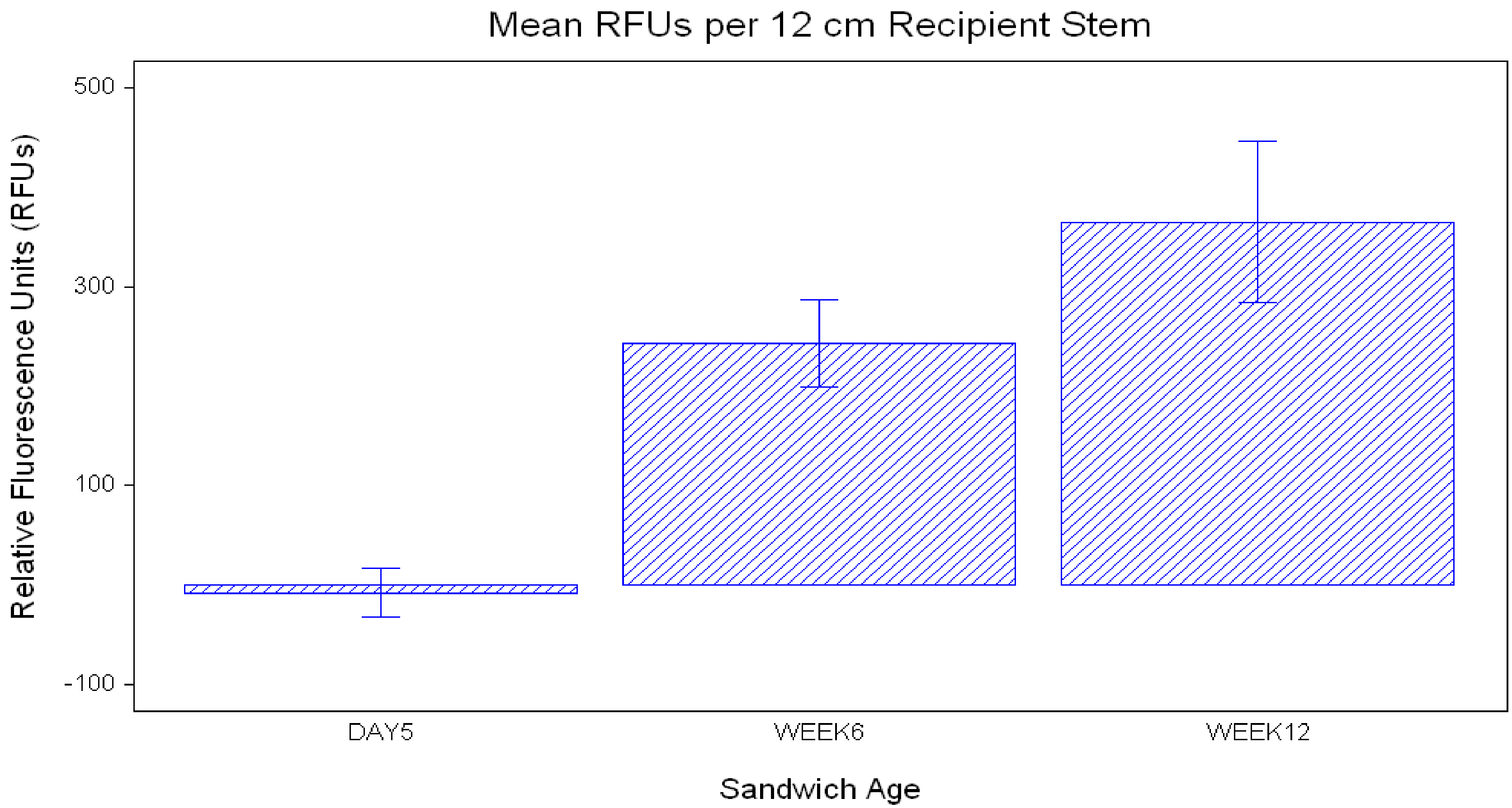

Figure A.6. Sandwich age treatment means expressed in relative fluorescence units per 12 cm of receiver stem. Statistically significance was detected between treatments by analysis of variance and LSD comparison of the means test.

Table A.31. Analysis of variance examining the effects sandwich age on rhodamine transfer to receiver stems. Rhodamine concentration calculated using relative fluorescence units per gram d.w. stem.

SOURCE	DF	SS	MS	F	P
Treatments	2	15813.2	7906.58	17.75	<0.0001
Error	28	12475.3	445.547		

Table A.32. Sandwich age treatment means and standard deviations calculated as relative fluorescence units per g d.w. of stem.

VARIABLE	MEAN	SIZE	STD DEV
DAY5	-2.6250	8	5.9545
WEEK6	33.719	16	19.950
WEEK12	61.829	7	32.294
TOTAL	30.687	31	21.108

Table A.33. Comparison of the means LSD test of sandwich age treatment means using relative fluorescence units per g d.w. receiver stem.

VARIABLE	MEAN	HOMOGENEOUS GROUPS
WEEK12	61.829	I
WEEK6	33.719	.. I
DAY5	-2.6250	 I

*All 3 means are significantly different from one another.
**Critical T value 2.084; rejection level 0.050

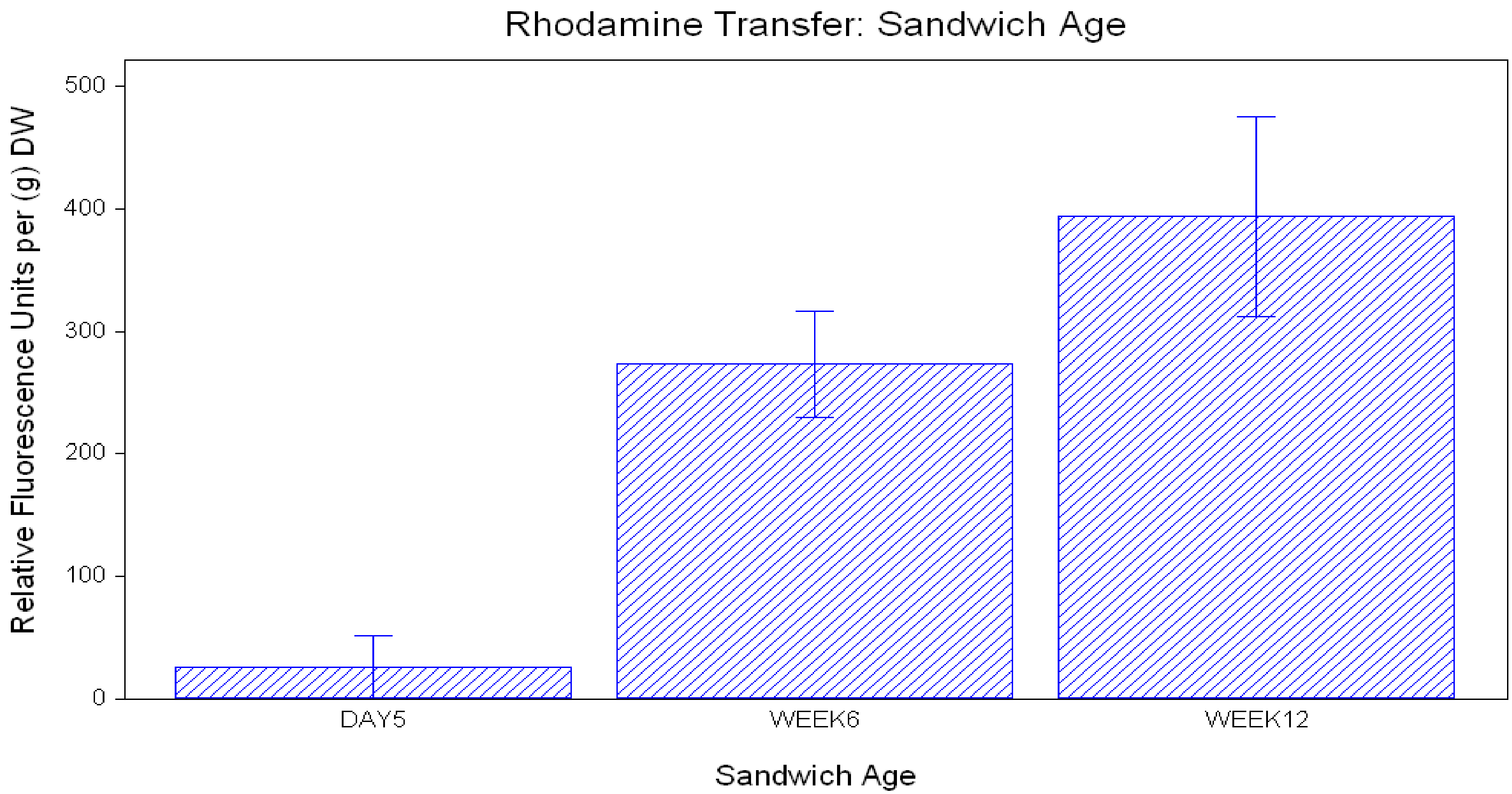

Figure A.7. Sandwich age treatment means expressed in relative fluorescence units per g d.w. of receiver stem. Statistically significance was detected between treatments by analysis of variance and all treatments using an LSD comparison of the means test.

Table A.34. Analysis of variance examining the effects of sandwich age on rhodamine transfer from donor plants to receiver stems. Percent receiver stem rhodamine [RG] concentration calculated by receiver [RG]/donor [RG]*100.

SOURCE	DF	SS	MS	F	P
Treatments	2	11231.9	5615.94	7.12	0.0032
Error	28	22088.9	788.889		

Table A.35. Sandwich age treatment means and standard deviations for percent transfer calculated by receiver [RG]/donor [RG]*100.

VARIABLE	MEAN	SIZE	STD DEV
DAY5	-6.5875	8	11.168
WEEK6	38.575	16	32.800
WEEK	1231.600	7	29.093
TOTAL	25.345	31	28.087

Table A.36. Comparison of the means LSD test of sandwich age treatment means calculating percent transfer using receiver [RG]/donor [RG]*100.

VARIABLE	MEAN	HOMOGENEOUS GROUPS
WEEK6	38.575	I
WEEK12	31.600	I
DAY5	-6.5875	.. I

*There are 2 groups in which the means are not significantly different from one another.
**Critical T value 2.084; rejection level 0.050

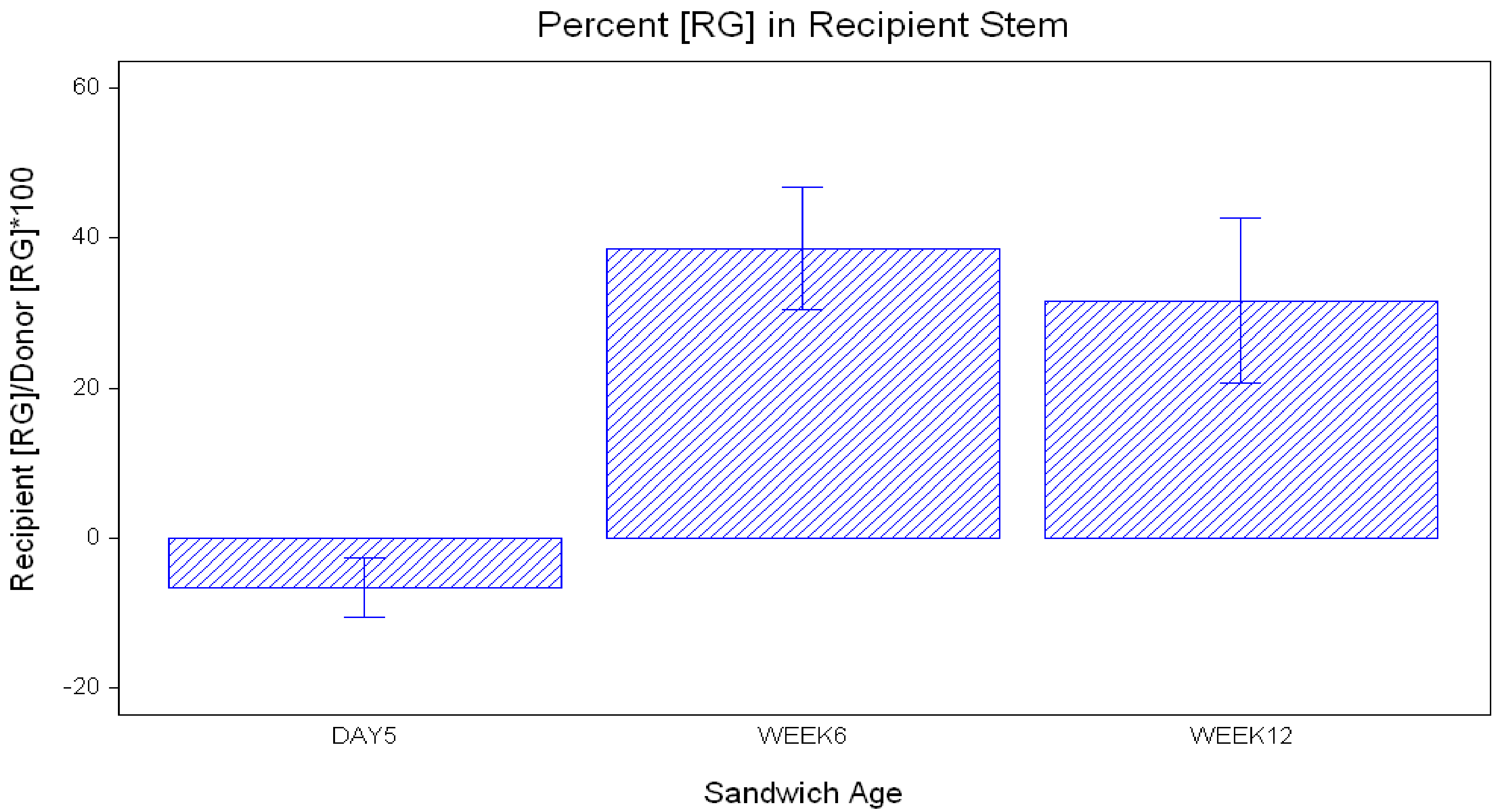

Figure A.8. Sandwich age treatment means . expressed in percent relative fluorescence units (RFU) per gram d.w. in receiver (*Desmanthus illinioensis*) stem versus with RFUs per gram in donor stems with ± SE. Statistical significance was detected between treatments by analysis of variance and LSD comparison of the means test.