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Do Novel Weapons that Degrade Mycorrhizal Mutualisms Explain Invasive Species Success?

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Do novel weapons that degrade mycorrhizal mutualisms explain invasive species success?

by

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An Abstract for a Thesis in
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ABSTRACT OF THESIS

Do novel weapons that degrade mycorrhizal mutualisms explain invasive species success?

Abstract:

Invasive plants often dominate novel habitats where they did not co-evolve with local species. Several hypotheses suggest mechanisms that explain increased exotic plant success, including 'novel weapons' and 'degraded mutualisms'. Japanese knotweed (*Fallopia japonica*) and European buckthorn (*Rhamnus cathartica*) are widespread plant invaders in North America that can dominate ecosystems. The goal of this study is to test whether these impacts are more consistent with novel weapons or degraded mutualism hypotheses. I examine tree seedling recruitment, (germination and initial survival) growth, (biomass) and mycorrhizal invasion (AMF content) as a function of *F. japonica* and *R. cathartica* root exudates. Given that species co-evolved with these invasive species may have compensatory mechanisms for the allelochemicals, I use arbuscular (AMF) and ectomycorrhizal (ECM) tree congeners that both co-occur and do not co-occur with the invasive species. My results suggest that novel weapons both attack the seedlings directly and indirectly degrade their mutualisms. Novel weapons imposed the greatest impact on *Ulmus* tree seedling germination as the root exudates significantly reduced germination in the *Ulmus* species that evolved in the absence of the invasive plants. However, the *Ulmus* species during later life stages (seedling survivorship and growth), appeared more dependent on mycorrhizal fungi, and *R. cathartica* degraded the AMF of *Ulmus* congeners. These results suggest that both novel weapons and degraded mutualisms help explain the success of these widespread invaders, and that the impacts

depend on life stage. Hence, successful species invasion may bring a suite of weapons rather than a single magic bullet.

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Introduction:

Invasive plants generally outcompete and displace native species (Levine et al. 2003; Spector & Putz 2006). The competitive advantages held by invasive species likely derive from several mechanisms, such as release from home range enemies, including specialist consumers, pathogens and parasites (Mack et al. 2000; Maron & Vila 2001; Levine et al. 2003; Keane & Crawley 2002; Mitchell et al. 2006). The reduced enemy load indirectly gives non-native species a greater competitive ability by exempting them from the negative burdens carried by the native competitors (Rabotnov 1982; Blossey & Nötzold 1995; Mallik & Pellisier 2000; Müller-Schärer et al. 2004). Non-native species also may possess direct advantages by bringing competitive mechanisms to which native competitors are not adapted, such as novel weapons and degraded mutualisms (Janos 1980; Callaway & Ridenour 2004; Stinson et al. 2006; Smith & Smith 2011; Smith & Smith 2012).

Plants weapons include phytotoxins that directly harm competing plants (Bais et al. 2003; Duke & Dayan 2006; Hale & Kalisz 2012). Some phytotoxins disrupt essential plant processes by targeting photosynthetic machinery, and/or the enzymes involved in respiration (Duke & Dayan 2006 Cipollini et al. 2012; Dallali et al. 2014). *Centaurea maculosa* (spotted knapweed) disrupts calcium signaling in the root meristem of competitors (Bais et al. 2003). Similar phytotoxins also can inhibit seed germination and seedling growth (Inderjit et al. 2008; Klionsky et al. 2011; Jessing et al. 2014).

Plants also may indirectly inhibit competitors by employing allelochemicals that attack their mutualist partners (Raguso 2008; Stinson et al. 2006; Cantor et al. 2011). For example, some phytotoxic chemicals deter competitor reproduction by masking or

overpowering attractive floral scents, thereby reducing pollinator visitation (Raguso 2008). Belowground, plants release anti-microbial allelochemicals that reduce competitor fungal mutualisms by inhibiting AMF spore germination (Schreiner & Koide 1993; Vierheilig et al. 2000; Stinson et al. 2007; Callaway et al. 2008; Cantor et al. 2011; Hale & Kalisz 2012).

Most woody plants require mycorrhizal colonization for germination, growth and/or survival (Nantel & Neumann 1992; Siqueira & Saggin-Junior 2001). Indeed, 90% of terrestrial plants form mycorrhizal associations (Smith & Read 2008). Mycorrhizal fungi increase plant nutrient and water absorption (Hardie & Leyton 1981; Sieverding 1981; Harley & Smith 1983; Chalot & Brun 1998; Leake et al. 2004;) in exchange for up to 20% of the carbon assimilated by plant photosynthesis (Johnson et al. 1997; Bago et al. 2000; Graham 2000; Högberg & Read 2006). Ectomycorrhizae (ECM) and arbuscular mycorrhizal fungi (AMF) have similar functions, but differ both morphologically and evolutionarily (Brundrett 2002). ECM filaments live within the plant roots, but only in the extracellular spaces, whereas AMF penetrate the cortical cells of the plants roots (Malloch et al. 1980; Smith & Read. 1997). ECM are made up of the more recently diverged higher fungi, whereas AMF originated much earlier, when plants were just getting a foothold in terrestrial habitats (Gehrig et al. 1996; Brundrett 2002).

Mycorrhizal mutualisms increase plant fitness compared to plants without colonized roots (Janos 1980; Koide & Dickie 2002). Mycorrhizae increase plant nutrient acquisition as the fungi 'scavenge' for soluble phosphorus and 'mine' for insoluble organic nitrogen (Ames et al. 1983; Plenchette, et al. 1983; Lambers et al. 2008; Smith & Smith 2012). Mycelial filaments promote a greater water scavenging ability that increase plant

drought tolerance (Hardie & Leyton 1981; Sieverding 1981; Leake et al. 2004; Stamets 2005; Allen 2007). Plants with intact fungal mutualisms also exhibit higher nitrogen assimilation during the recovery period after water stress (Panwar 1992; Subramanian & Charest 1995). Additionally, with mycorrhizae, plants can allocate more nutrients, water and energy towards reproductive effort. Mycorrhizal hosts generally have larger flowers, more flowers, higher nectar sugar content, and an increased number of active stamens (Gange & Smith 2005; Varga & Kytöviita 2010; Aguilar-Chama & Guevara 2012).

The allelopathic degradation of competitor fungal mutualisms may provide a decided competitive advantage (Schreiner & Koide 1993; Vierheilig et al. 2000; Stinson et al. 2007; Cantor et al. 2011; Hale & Kalisz 2012). Moreover, plants that do not require obligate mycorrhizal fungi may be more likely to use traits that degrade mycorrhizal fungi (Bais et al. 2003; Stinson et al. 2007). For example, garlic mustard (*Alliaria petiolata*) is a highly invasive species in North America (N.A.) that comes from a lineage of plants that do not require mycorrhizae for germination or nutrient acquisition (Janos 1980; Smith & Reed 1997; Brundrett 2002; Stinson et al. 2007; Smith & Read 2008; Smith & Smith 2011; Smith & Smith 2012). In turn, *A. petiolata* root exudates (glucosinolates, flavonoids, and allyl isothiocyanate) can inhibit fungal spore germination by up to 57% and, as a result, the number of mycorrhizal soil propagules decrease when *A. petiolata* is present (Herrera et al. 1993; Requena et al. 1996; Stinson et al. 2007; Callaway et al. 2008; Cantor et al. 2011). The lowered mycorrhizal potential in invaded soils gives *A. petiolata* a competitive edge against mycorrhizal dependent individuals by creating a fungal inhibitory zone (Stinson et al. 2007; Callaway 2008).

Another Old World secondary compound, emodin, is found in *Fallopia japonica*, (Japanese knotweed) and *Rhamnus cathartica* (European buckthorn). The allelochemical emodin is a widespread secondary compound found in 17 plant families (Izhaki 2002). Emodin is a multifunctional compound that provides a competitive edge by interacting with surrounding fauna and flora. Emodin helps the plant compete by deterring herbivory from insects and birds (Trial & Diamond). Emodin also inhibits seedling growth (Inoue et al. 1992) and root/shoot development (Hasan 1998; Tucker 2016). The germination, growth, and survival of flowering understory shrubs becomes reduced when a non-native species containing emodin infiltrates a new habitat (Klionsky et al. 2011, Sera 2012). Chemically, emodin can alter soil dynamics, including the accumulation of soil nitrogen, and increased soil pH (Trial & Dimond 1979; Francis et al. 1998; Tsahar et al. 2002; Heneghan et al. 2006). Many studies show how emodin directly interacts with neighboring plant competitors, but its indirect effects remain unclear.

Fallopia japonica may have a similar effect to *A. petiolata,* indirectly competing with plants by degrading their mycorrhizae. Like *A. petiolata,* emodin containing *F. japonica* does not form mycorrhizal mutualisms (Schnitzler & Muller 1998). The roots of most non-mycorrhizal plants have more recently adapted, higher functioning root qualities (Skene 1998; Brundrett 2002). Many non-mycorrhizal plant roots have surpassed their ancestral condition of requiring mycorrhizae, accessing pools of nitrogen and phosphorus without engaging in expensive fungal mutualisms (Lambers et al. 2008). Plants that don't require mycorrhizae should be more likely to degrade the fungal mutualisms of nearby plant competitors. Non-mycorrhizal plants with these phytotoxins

that degrade mutualisms reduce neighboring plant fitness, without negatively impacting their own nutrient and water acquisition.

Unlike *A. petiolata* and *F. japonica*, *R. cathartica* is an AMF species (Godwin 1943; Knight 2006). AMF dependent plants may still degrade mutualisms, but only selectively towards ECM plant species. Plant allelochemicals that can degrade their own fungal symbionts should not evolve as competitive mechanisms, as implementing degradative mutualistic compounds would hinder the plants own fitness. Natural selection should only provide plants with competitive mechanisms that do not have a high fitness cost.

The goal of this study is to investigate the allelochemical effect of two Eurasian invasive species, *R. cathartica* (shrub) and *Fallopia japonica* (herb) on two globally distributed tree genera, *Betula* (ECM) and *Ulmus* (AMF). Both invasive species contain the secondary compound emodin, which is a potent allelochemical that reduces competitor plant fitness (Inoue et al. 1992; Nishimura & Mizutani 1995; Tucker 2016). Emodin has a direct impact on competitors (Klionsky et al. 2011; Sera 2012; Hasan 1998; Tucker 2016; Inoue et al. 1992) and may have an indirect effect on plant competitors by reducing mycorrhizal fungi, because it has been shown to inhibit spore germination of parasitic fungi (Singh et al. 1992). If the invasive allelochemicals act only as direct novel weapons, I expect the tree seedling mycorrhizal communities to remain unaffected while seedling recruitment and performance is reduced between tree seedlings and invasive exudates that did not share co-evolutionary distributions. Alternately, if the impact mechanism is degraded mutualisms, I expect that *R. cathartica* (AMF) will only impact tree performance through reducing the ectomycorrhizal invasion in *Betula* (ECM)

whereas *F. japonica* (non-mycorrhizal) will impact both *Betula* and *Ulmus* (AMF) functioning by reducing fungal-root colonization.

Methods

Tree seedling germination, survival and growth were assessed as a function of species identity (three *Ulmus* congeners and three *Betula* congeners), invasive species (*F. japonica* and *R. cathartica*) and fungicide.

Study species

The effects of *R. catharctica* and *F. japonica* allelochemical exudates were tested using three *Betula* congeners and three *Ulmus* congeners (Table 1). The three AMF tree species were *Ulmus alata, U. parvifolia* and *U. minor*. *Ulmus alata* (winged elm) is a medium-size species growing to 12-24m tall, which is indigenous to eastern N. A. (Little 1980). *Ulmus parvifolia* (Chinese elm) is a medium-size species reaching a height of 10-20m, that has a wide-ranging, East Asian distribution (Little 1980). *Ulmus minor* (the field elm) is a large species growing up to 30m, found throughout Europe (Richens 1983) (Table 1). All of the selected tree species have similar moisture and nutrient requirements (Little 1980; Coyle et al. 1982; Richens 1983; Atkinson 1992; Bu et al. 2007).

The three ECM study tree species were *Betula pubescens*, *B. nigra* and *B. davurica*. *Betula pubescens* (European white birch) is a medium-size tree growing 10-25m in height, with a wide European distribution (Atkinson 1992). *Betula nigra* (black birch) is a large birch species growing up to 25-30m, that is native to eastern North America (Coyle et al. 1982). *Betula davurica* (Asian black birch) is a medium-size tree,

reaching 12-15m in height with a wide-ranging East Asian distribution (Bu et al. 2007) [Table 1].

Germination experiment

The 14-week germination experiment (July-October 2015) was carried out at the Dorsheimer Laboratory/Greenhouse (State University of New York at Buffalo, Buffalo, NY). 650-700 grams of soil media were added to 180, 25cm tall tree seedling planters (Stuewe and Sons, Tangent, Oregon USA). The mycorrhizal soil media was created to be nutrient poor, and coarse. (Table 2). The soil media contain the spores of eleven species of mycorrhizal fungi. Four generalist arbuscular fungi (*Glomus intraradices, Glomus mosseae, Glomus aggregatum, Glomus etunicatum*) and seven generalist ectomycorrhizal species (*Rhizopogon villosulus, Rhizopogon luteolus, Rhizopogon amylopogon, Rhizopogon fulvigleba, Scleroderma cepa, Scleroderma citrinum, Pisolithus tinctorius*).

Pre-experimental germination rates for each of the 6 tree species differed from the germination rates provided by the seed distributor (Sheffield's Seed Co. Locke, NY), so the number of seeds planted in each of the planters was adjusted (Table 1). The greenhouse temperature was 25ºC throughout the duration of the experiment. The seeds/seedlings were watered twice a day. The mesocosms were checked for germination weekly.

After 14 weeks of plant growth, the tree juveniles were harvested and one gram of living root material from each germinated individual was prepared for root staining. The rest of the plant was rinsed of all soil material, placed into a labeled paper bag and dried in a drying oven at 60ºC for 5 days before weighing.

Fungicide/Exudate collection and processing

Soil mesocosms designated for fungicidal treatment received 14 mg of fungicide (Captan 50WP) per gram of soil (Table 3). This amount was suggested by the manufacturer (Bonide products, Oriskany, NY USA) and pre-experimental testing indicated that it successfully inhibited fungal spore germination. Root material from both *R. cathartica* and *F. japonica* were collected from Tift nature preserve, (Buffalo, New York USA). The roots were washed thoroughly and dried for 3-5 days at 60°C. *Fallopia japonica* roots required a longer drying time because of their bulbous, robust characteristics. Dried roots were pulverized into a fine, uniform powder and soil mesocosms received ten grams of powdered root material of either *F. japonica* or *R. catharctica* (Table 3).

AMF colonization assay

The staining procedure was slightly modified from Phillips & Hayman (1970). The rinsed one gram of fresh root material was put into labeled test tubes held upright in a test tube holders, then placed in a hot water bath with 100°C water. 10-15 milliliters of 10% KOH was placed in each tube, and was heated in the water bath for 25 minutes. The KOH solution becomes a darker brown color, as the cytoplasmic contents of the plant cells are removed. The root material was then rinsed 4-5 times, and placed in newly labeled test tubes. 2% HCl solution was added for 15-20 minutes to ensure the acidification of roots so the stain will chemically bind properly.

The stain is prepared by combining water, glycerin, and lactic acid in 1:1:1 ratio(v/v/v). The stain is completed when it contains 0.05% acid fuchsin. New labeled

tubes containing the cleared, acidified roots with the mycorrhizal stain were refrigerated for 24 hours. The root material was strained, rinsed and stored in DI water for a week, so the excess stain can leach out of the roots. This creates a stronger contrast between fungal and plant cells.

To quantify arbuscular, vesicular, and hyphal colonization I used the objective crosshair technique, identical to the procedures described in McGonigle et al. (1990). The unknown prepared AMF tree roots were placed on microscope slides, and focused using an Olympus CX31 compound microscope (Shinjuku, Tokyo, Japan). On the eyepiece of the compound microscope, two intersecting perpendicular lines (crosshairs) were drawn. Once the specimen was focused, five random root segments were selected. At each of the five segments, ten fields of view were analyzed, tallying a total of 50 mycorrhizal observations for each root sample.

With each field of view, the slide and or eyepiece was manipulated so that one of the two crosshairs dissected the root widthwise. If the crosshair cut across an arbuscule, I increased the arbuscule tally for that sample by one. If a crosshair intersected more than one arbuscule in a single field of view, it was still only tallied once. The same is true for crosshairs traversing vesicles, and hyphae. If a crosshair overlapped both an arbuscule and a vesicle, a tally was marked for both. However, since hyphae co-occur with the other two fungal structures so frequently, when they did appear with either a vesicle or arbuscule, the vesicle or arbuscule was accounted for, while the hyphae was not. Just as importantly, fields of view without any fungal formations where tallied as mycorrhizae absent.

Data analysis

Plant germination and survival were analyzed using generalized linear models (GLM) assuming a binomial error distribution with seedling species, invasive species and fungicide as categorical treatments. Germination was calculated as seedlings emerged by week six. Survivorship was calculated as week 14 survivors from those that germinated at week 6. The coefficients for the fitted GLM models were estimated using analysis of deviance (ANODEV) with Chi-square tests. Collinearity was tested using the variance inflation function in the package 'car' (Fox & Weisberg 2011). The data also were checked for overdispersion, ($\varphi > 1.5$) and corrected when needed using quasi error distributions. Coefficients were considered significant with *p-values* of *<0.05*, whereas *p-values* <0.10 were considered as marginally significant (*sensu* Hurlbert & Lonbardi 2009). All data were analyzed using R statistical software (R Development Core Team 2016).

Given that the biomass data (g) were highly skewed and could not include numbers below zero, growth was analyzed using a GLM with a Poisson error distribution and fitted using ANODEV. The mycorrhizal data (arbuscules, vesicles, hyphae) all were analyzed using GLM models with a binomial proportion (presence of fungal structure/50 samples) and fitted using ANODEV.

Pearson's correlation coefficient was used to examine correlation between the three fungal indicators and plant growth (biomass). Based on the correlation results, a linear regression model was used to test the relationship between mycorrhizal vesicles and plant biomass.

Results:

Germination

Overall, germination was very low (21%). *Betula nigra* and *B. pubescens* both had a germination rate of 12%. *Ulmus alata* and *Betula davurica* both had zero seedlings germinate. *Ulmus parvifolia* (44%) and *U. minor* (80%) had the highest germination rates. A marginally significant species x invasive interaction term indicated a species-specific effect of invasive root exudates on tree germination (Table 4). Both *Betula* spp. were unaffected by *R. cathartica* and *F. japonica* root exudates (Fig. 1); however, *U. minor* (Europe) germination dropped significantly with *F. japonica* (Asian) exudates and was unaffected by *R. cathartica* (Europe) [Fig. 1]. Conversely, *U. parvifolia* (Asia) germination dropped significantly with *R. cathartica* (Europe) exudates and was unaffected by *F. japonica* (Asian) phytotoxins (Fig. 1). Tree seedling germination was unaffected by the fungicide treatment, and there was no fungicide x invasive interaction effect (Table 4).

Survivorship and growth

Once a seed germinated, 71% of the seedlings lived to harvest at 14 weeks. There was marginally significant lesser seedling survival with the fungicide treatment than control (Fig. 2), and survivorship did not differ between species, invasive species or interactions (Table 5). Tree species growth (dry biomass) was marginally significantly higher for *Ulmus* than *Betula* spp. (Fig. 3a), and significantly lesser with invasive root exudates than control (Fig. 3b, Table 6).

Mycorrhizal data

Given that the *Betula* germination rates were so low, mycorrhizal analysis only was conducted on the AMF *Ulmus* species. Arbuscular presence decreased significantly in the presence of *R. cathartica* root exudates for both *Ulmus* species (Fig. 4), but fungicide and fungicide x invasive had no effect (Table 7). Vesicle presence decreased marginally significantly with both invasive root exudates (Fig. 5a) and decreased significantly with fungicide (Fig. 5b, Table 8). Fungal hyphae decreased significantly with fungicide but were unaffected by invasion and invasion x fungicide (Table 9).

The mycorrhizal parameters (arbuscules, vesicles and hyphae) were moderately correlated (r = 0.40 to 0.50), and vesicle presence correlated strongest with plant biomass (r = 0.64), and plant biomass increased significantly (*Estimate* = 0.026, *SE* = 0.174, *t-value* = 3.584, *p-value* = 0.002; r^2 = 0.29) with increased vesicle presence (Fig. 6).

Discussion:

Exotic species may employ a multi-prong attack on novel competitors -- both directly and indirectly by reducing their mutualist partners. The results presented here suggested that tree seeds resisted familiar direct allelopathic weapons from plants species with which they co-evolved; the same weapons devastated seed germination when introduced to novel tree species. These results are consistent with the novel weapons hypothesis, indicating that species evolve compensatory mechanisms to resist competitive weapons in their native communities. Once established, however, the congener effect faded and initial tree seedling survivorship was unaffected by invasive species. Instead, seedling growth decreased with either invasive species, but *R. cathartica* treatments had an

indirect effect on *Ulmus* sp. due to its allelopathic degradation of symbiotic fungi – a result consistent with the degraded mutualism hypothesis. Overall, these results support both novel weapons, and degraded mutualisms hypotheses.

Interactions between foreign allelochemicals and the initial life stages of *Ulmus* species showed a direct competitive mechanism from both invasive species. The germination of European *U. minor* was unaffected by European *R. cathartica*, but significantly reduced by Asian, *F. japonica* root exudates (Fig. 1). Similarly, the germination of Asian *U. parvifolia* was reduced by unfamiliar *R. cathartica* allelochemicals, but unaffected by the exudates of a co-evolved *F. japonica* (Fig. 1). Indirect competitive mechanisms only were apparent between *Ulmus* species and *R. cathartica*. Arbuscular and vesicular colonization were reduced in *Ulmus* sp. with the addition of *R. cathartica* root exudates. Only vesicular formation was reduced when *Ulmus* sp. interacted with *F. japonica* allelochemicals, but vesicle formation alone is a weak indicator of a mycorrhizal mutualism because some mycorrhizae do not form vesicles inside plant roots. (Nicolson et al. 1968). The reduction of both arbuscular and vesicular formation indicated degraded mutualisms.

Emodin containing plants directly compete with non-native flora by inhibiting germination; a plant response implemented by both invasive species (Fig. 1). Chemically, emodin disrupts respiration and root meristem signaling, (Cipollini et al. 2012; Dallali et al. 2014) which may ultimately prevent seed germination. Novel weapons that do not have a direct effect on plant competitors can remain effective by indirectly attacking competitors through their mutualisms. *R. cathartica* use a combination of direct and indirect competitive mechanisms to ultimately limit the germination and growth of *Ulmus*

spp. These European exudates reduced arbuscular colonization and growth in the Ulmus congeners, regardless of their native distributions. This pattern occurred most likely because only one mycorrhizal community was used.

Callaway (2008) revealed that mutualistic fungi evolve resistance towards plant allelochemicals. Mycorrhizae in N.A. soils conditioned with European *A. petiolata* had a significantly lower fungal spore germination, spore count and AMF root invasion compared to the fungal communities in European soils. After studying the compensatory traits of different mycorrhizal communities, the findings in Callaway (2008) suggest that arbuscular mycorrhizal fungi evolve resistance against plant allelochemicals, when co-evolution occurs. The N.A. fungal communities without compensatory mechanisms against European *A. petiolata* become reduced, further supporting novel weapons hypothesis.

In supplementing Callaway (2008), these results suggest that the 4 species of AMF used in this study have not evolved compensatory mechanisms against novel weapons from Asia and Europe. Additionally, this study revealed that the three *Ulmus* congeners are also without evolved compensatory mechanisms against indirect phytotoxic mechanisms. These tree species lack the ability to maintain their own mycorrhizal communities when facing a plant competitor which engages in degraded mutualisms.

Competition drives species distributions, and may help describe the success of plant species invasion. The data presented here suggest that the success of two invasive plants, *F. japonica* and *R. cathartica*, may depend on how native flora and their fungal mutualists respond to novel allelochemicals. Plant species that co-evolved with these

plants appeared to have evolved compensatory mechanisms against direct, (plant targeted) phytotoxins, but their mutualist fungi did not. These results suggest that, rather than a single magic bullet, invading plants may employ a multi-prong attack.

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Table 1: The study species, the geographical region they evolved from, their general mycorrhizal association and the number of seeds planted for each species.

Plant Species	Native range	Mycorrhizae	# of seeds/planter
Betula pubescens	Europe	ECM	25
Betula davurica	Asia	ECM	16
Betula nigra	North America	ECM	20
Ulmus minor	Europe	AMF	8
Ulmus parvifolia	Asia	AMF	9
Ulmus alata	North America	AMF	6
Fallopia japonica	Asia	None	None
Rhamnus cathartica	Europe	AMF	None

Table 2: Composition of soil media.

Soil media ingredient	%
Mycorrhizal inoculated growth mix	46
Perlite	37
Vermiculite	9
Peat moss	8

Table 3: Overview of the five treatments used.

Treatment	Description
European Buckthorn	Ten grams of macerated buckthorn roots mixed into the soil.
Japanese Knotweed	Ten grams of macerated knotweed roots mixed into the soil.
Control	No manipulation of soil media.
Fungicide	14 milligrams of fungicide/gram of soil.
European Buckthorn + Fungicide	Buckthorn root and fungicide treatment mixed into the soil.
Japanese Knotweed + Fungicide	Knotweed root and fungicide treatment mixed into the soil.

Table 4: Analysis of deviance of germination (%) as a function of invasive root exudates/fungicide and their interactions.

Treatment	***df***	***Deviance***	***Res. dev.***	***p-value***
Invasion	2	4.025	123.16	0.13364
Fungicide	1	1.9	121.26	0.1681
Species:Invasion	6	11.657	109.6	0.07007
Invasion:Fungicide	1	0.437	109.17	0.50849

Table 5: Analysis of deviance of survival (%) as a function of invasive root exudates/fungicide, and their interactions.

Treatment	***df***	***Deviance***	***Res. dev.***	***p-value***
Invasion	2	0.4675	39.125	0.79155
Fungicide	1	3.471	35.654	0.06245
Species:Invasion	4	7.0097	28.645	0.13538
Invasion:Fungicide	1	2.4483	26.196	0.11765

Table 6: Analysis of deviance of plant growth (dry weight) as a function of invasive root exudates/fungicide and their interactions.

Treatment	***df***	***Deviance***	***Res. dev.***	***p-value***
Invasion	2	1738.61	2743.2	0.008145
Fungicide	1	73.73	2669.4	0.523
Species:Invasion	3	104.97	2564.5	0.900807
Invasion:Fungicide	1	81.84	2482.6	0.500972

Table 7: Analysis of deviance of arbuscular colonization (%) as a function of invasive root exudates/fungicide and their interactions.

Treatment	***df***	***Deviance***	***Res. dev.***	***p-value***
Invasion	2	48.149	161.48	0.05082
Fungicide	1	19.954	141.53	0.11608
Invasion:Fungicide	1	21.642	119.88	0.10172

Table 8: Analysis of deviance of vesicular colonization (%) as a function of invasive root exudates/fungicide and their interactions.

Treatment	***df***	***Deviance***	***Res. dev.***	***p-value***
Invasion	2	5.1581	22.684	0.07585
Fungicide	1	7.4766	15.207	0.00625
Invasion:Fungicide	1	0.1349	15.072	0.71337

Table 9: Analysis of deviance of hyphal colonization (%) as a function of invasive root exudates/fungicide and their interactions.

Treatment	***df***	***Deviance***	***Res. dev.***	***p-value***
Invasion	2	2.987	128.68	0.2246
Fungicide	1	51.533	77.15	0.0001
Invasion:Fungicide	1	1.079	76.07	0.2988

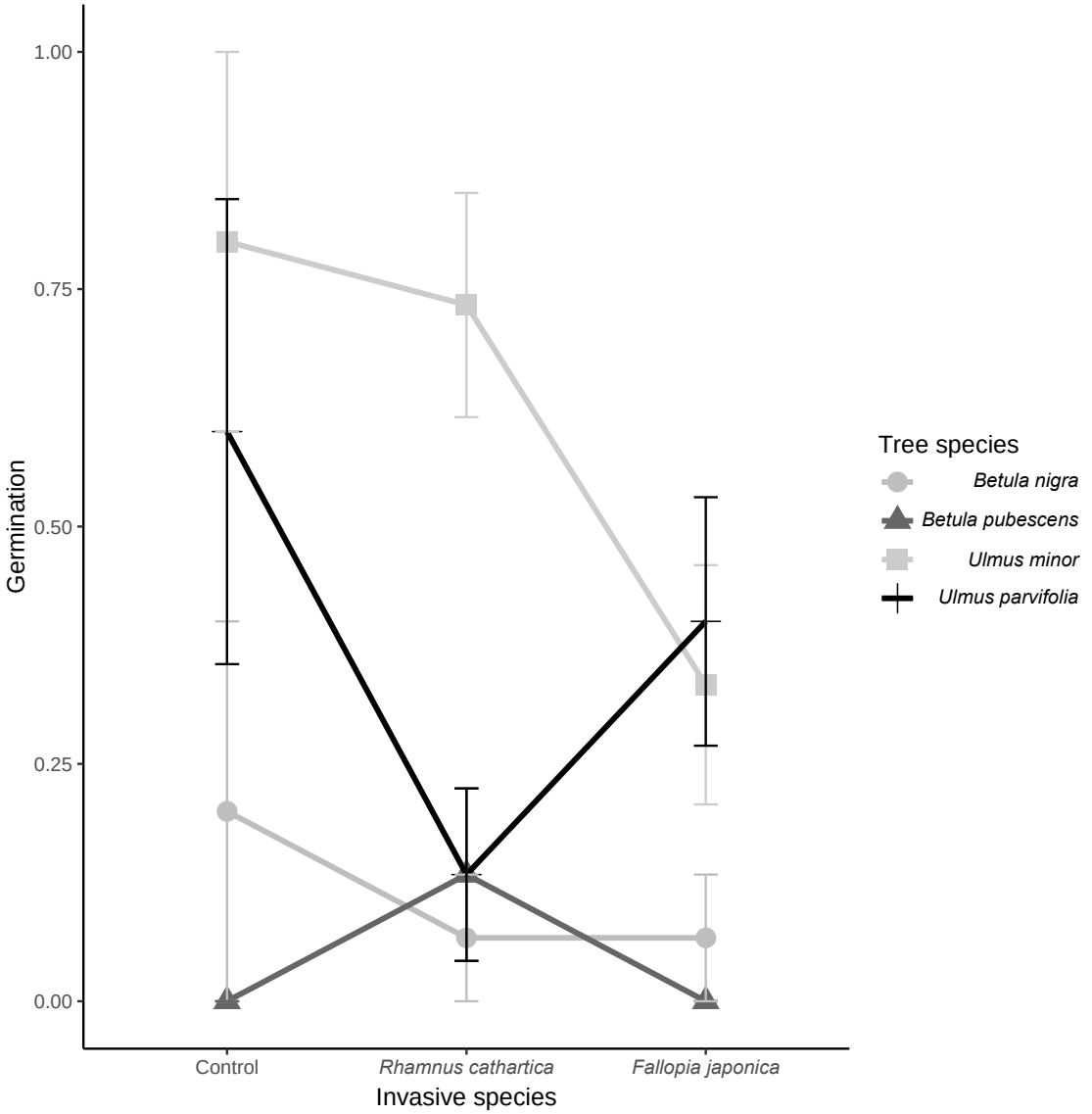

Fig.1. Interaction plot for species x invasive impacts on germination. A marginally significant interaction indicated that the effects of the individual invasive species root exudates were species specific on tree seed germination. *Betula* spp. appeared unaffected by treatments, but these effects may have been masked by low germination rates. For the *Ulmus* species that evolved in the absence of the invasive plants root exudates, germination was significantly reduced. When either *Ulmus* species encountered root exudates evolving within their native range, germination was unaffected.

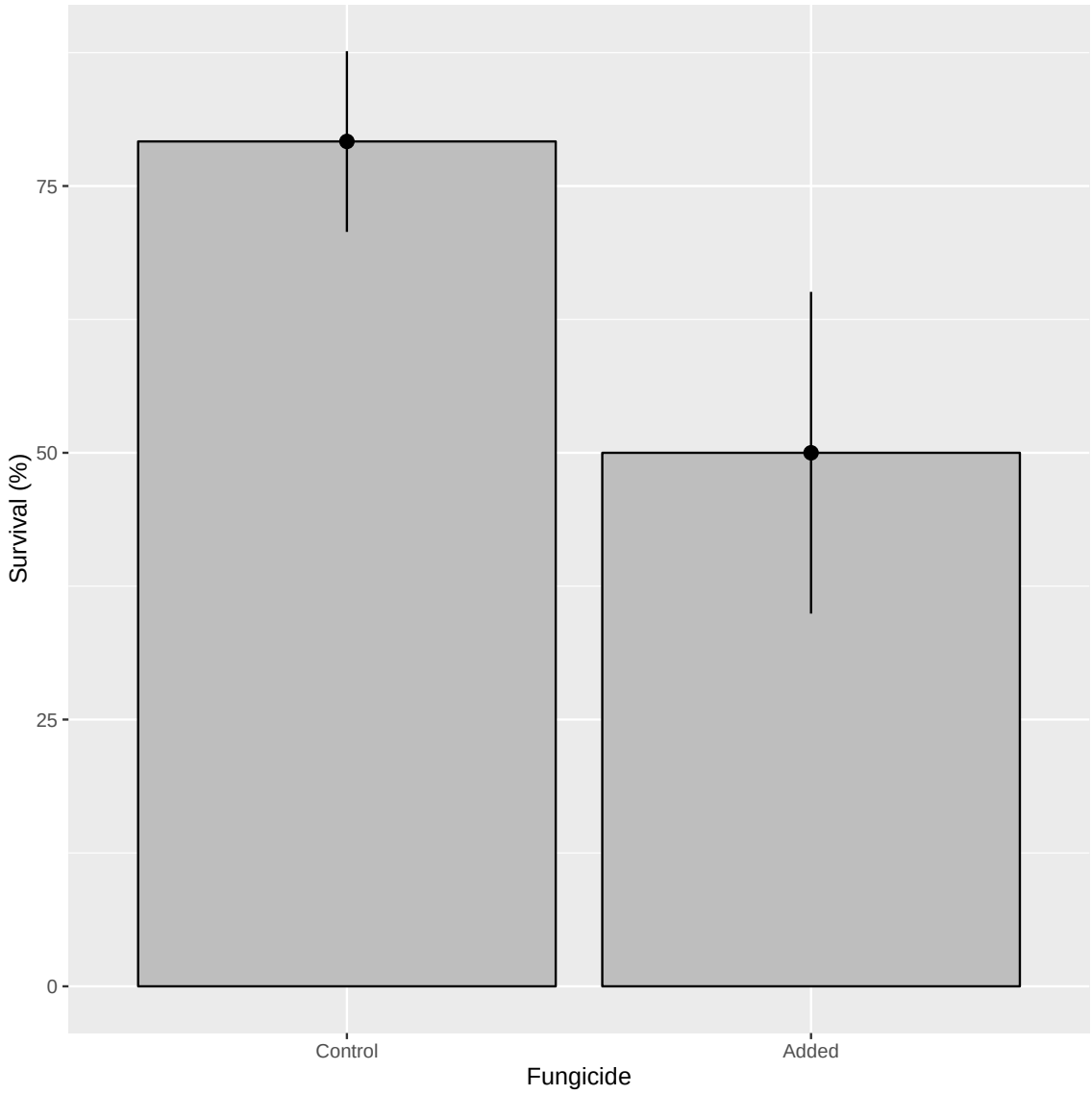

Fig.2. Plant survival as a function of fungicide. Fungicide decreased tree seedling survival.

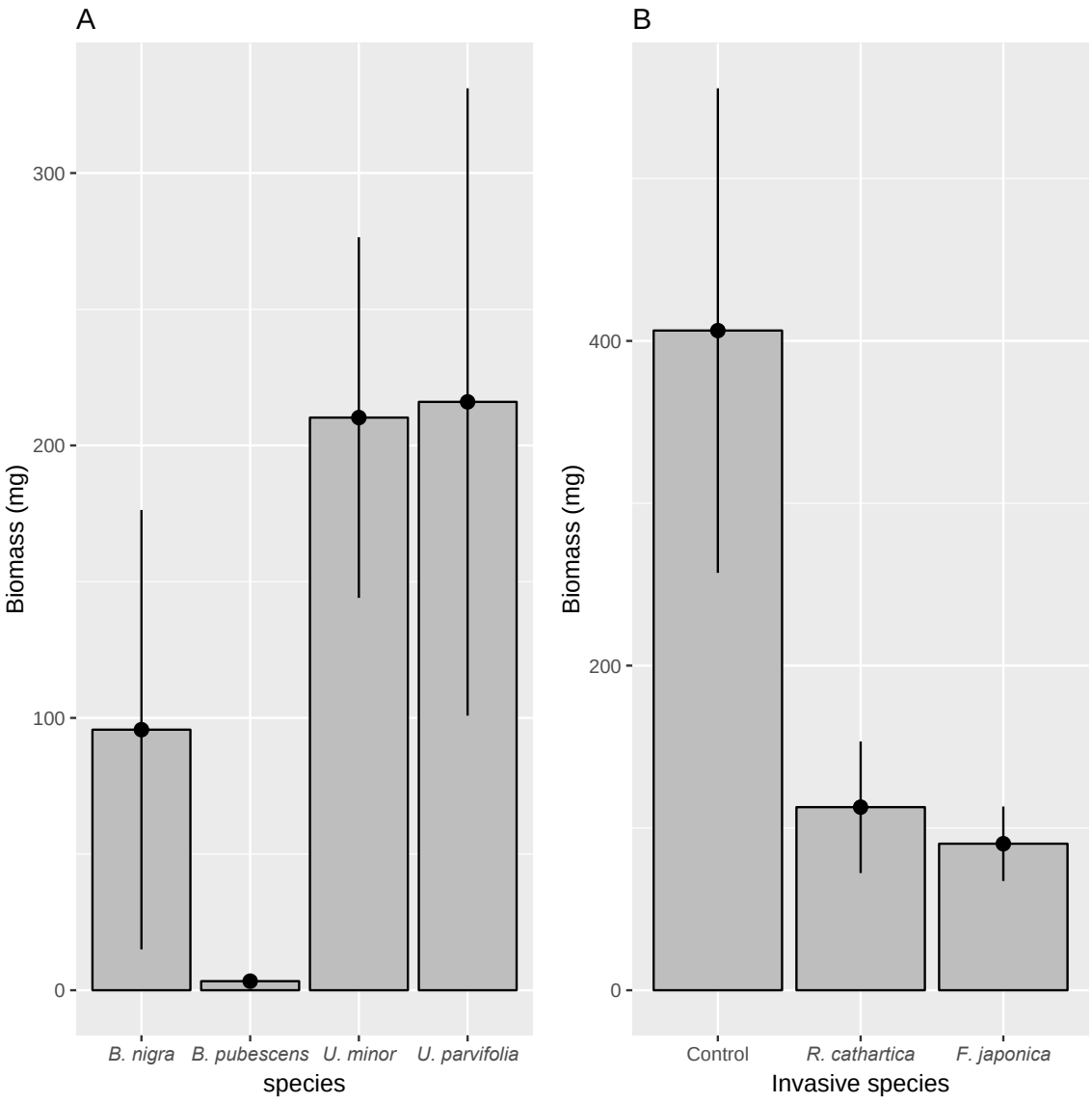

Fig. 3. Tree seedling growth (biomass at end of 14-week experiment). *Ulmus* spp. grew much more than *Betula* spp, (A) and both invasive plant species inhibited seedling growth in all tree species (B).

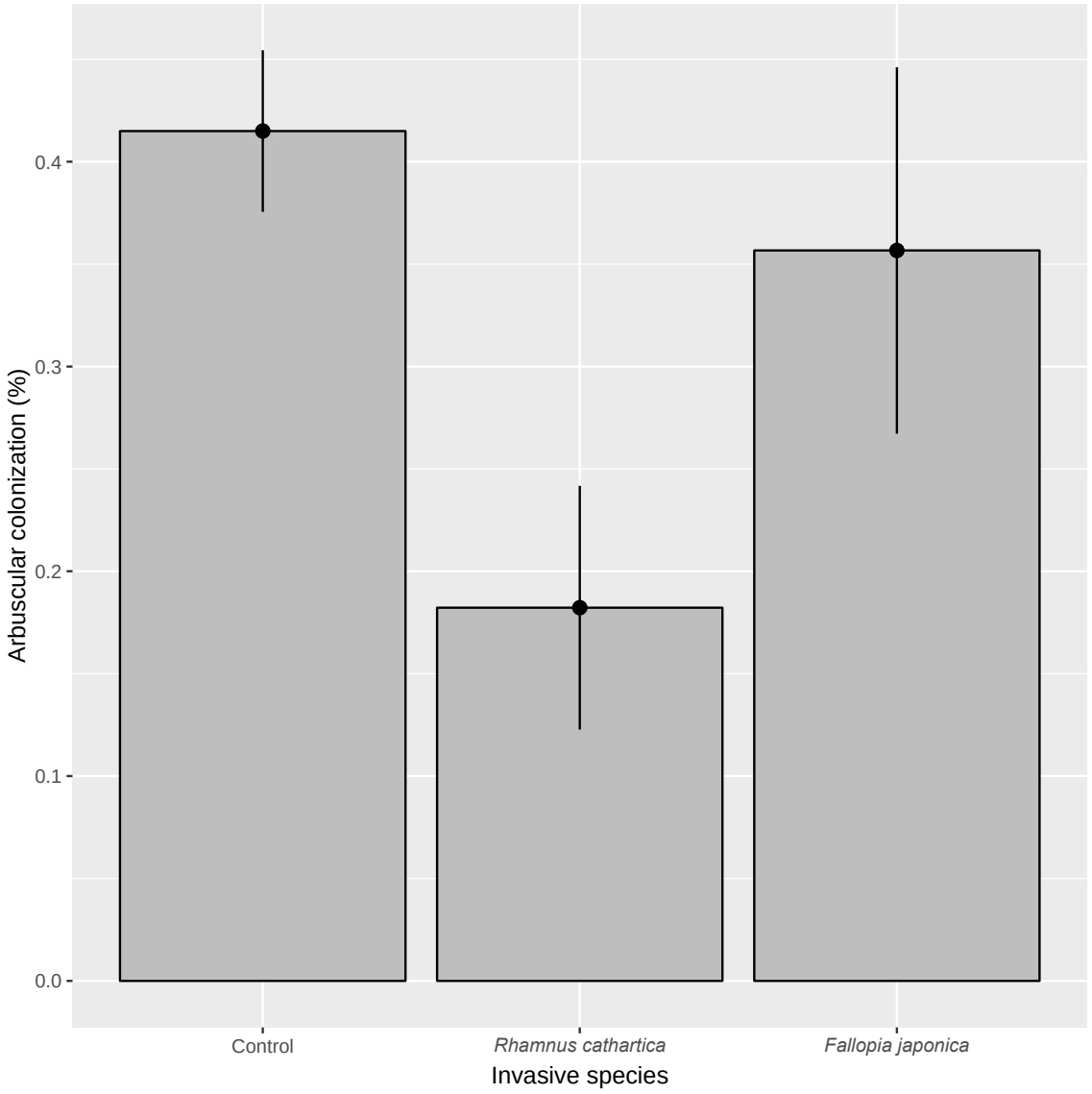

Fig. 4. Arbuscular colonization was reduced in *Ulmus* spp. with European invasive treatment. Japanese invasive treatment resulted in no significant change in *Ulmus* spp. arbuscular colonization.

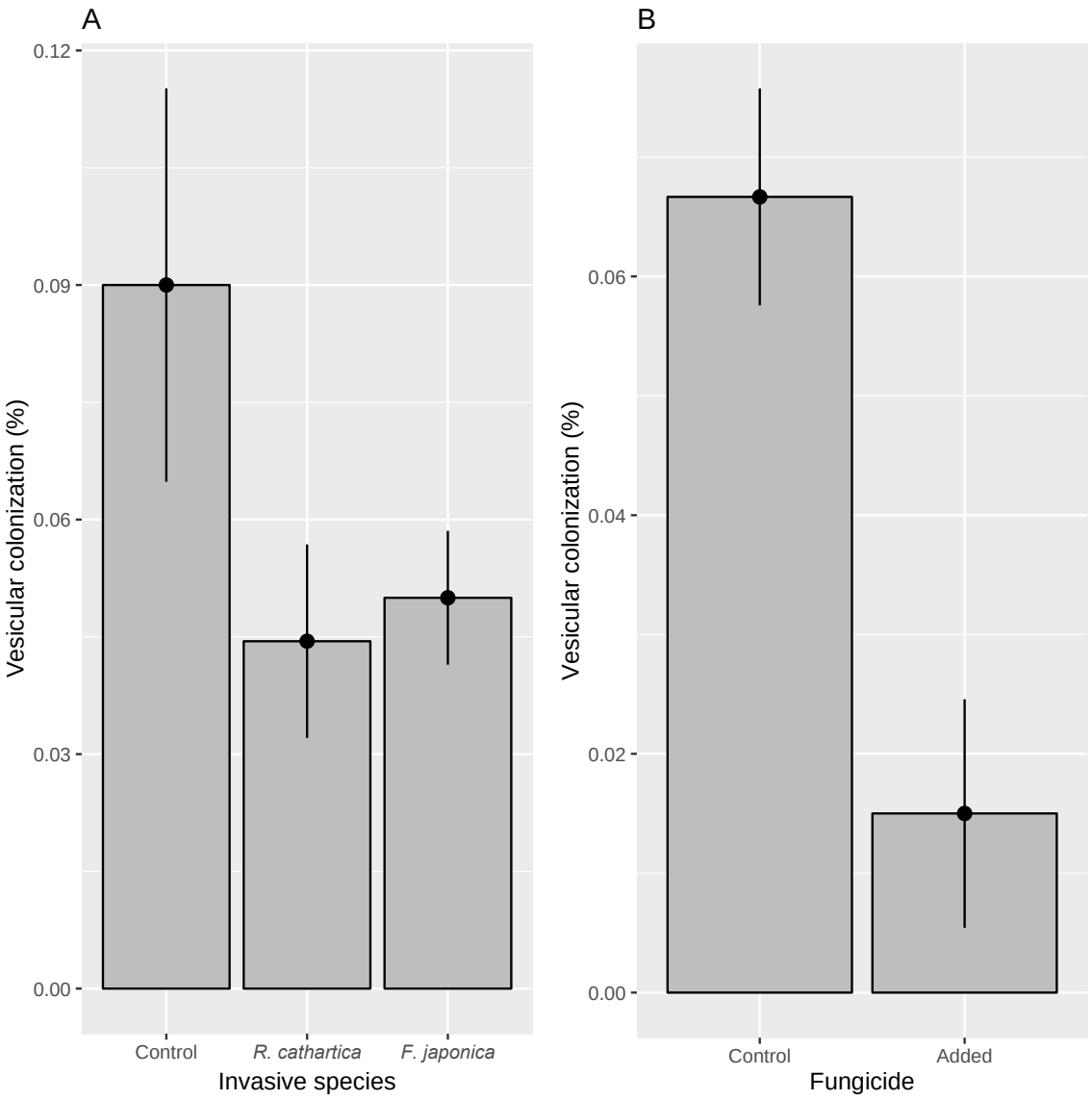

Fig.5. Both invasive plant species reduced vesicle formation in *Ulmus* spp. coefficient as a function of the invasive root treatments (A). Fungicidal treatments reduced vesicle colonization in *Ulmus* spp. (B).

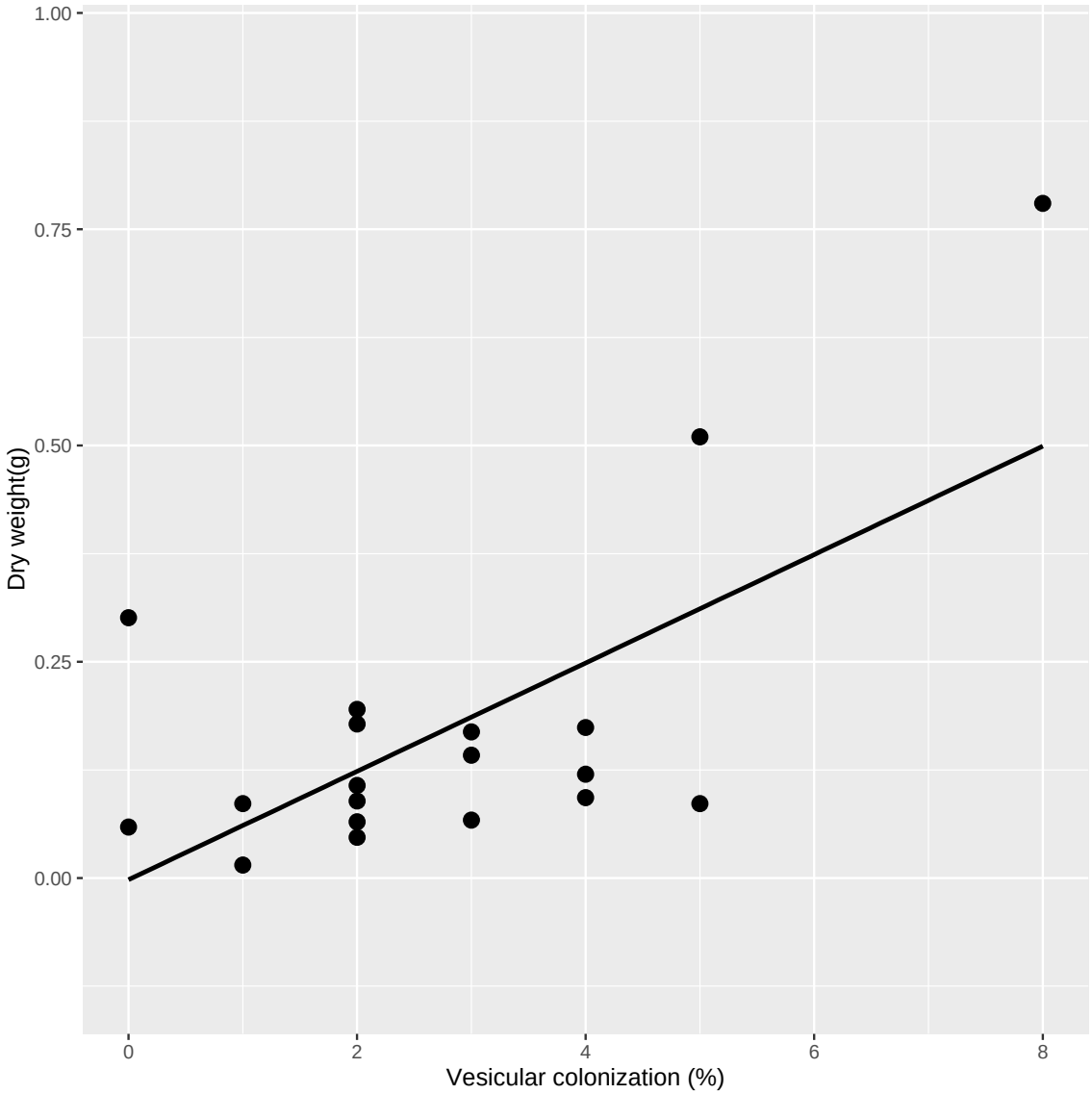

Fig. 6. *Ulmus* spp. dry mass increase as a function of vesicle colonization percent. Vesicle colonization best predicted plant weight (*p-value = 0.00229*, R^2 = *0.4304*, *std. error = 0.017480*). Plants with more fungal storage structures had larger mass. Vesicles indicate a well-established relationship between plant and fungi.