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Soil properties and actinorhizal vegetation influence nodulation of *Alnus glutinosa* and *Elaeagnus angustifolia* by *Frankia*

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Abstract

Nodulation (mean number of nodules per seedling) was 5 times greater for *Elaeagnus angustifolia* than for *Alnus glutinosa* overall when seedlings were grown in pots containing either an upland or an alluvial soil from central Illinois, USA. However, the upland Alfisol had 1.3 times greater nodulation capacity for *A. glutinosa* than for *E. angustifolia*. The presence of *A. glutinosa* trees on either soil was associated with a two-fold increase in nodulation capacity for *E. angustifolia*. Nodulation increases for soils under *A. glutinosa* were obtained for *A. glutinosa* seedlings in the Alfisol, but decreased nodulation for *A. glutinosa* seedlings occurred in the Mollisol. Greatest nodulation of *E. angustifolia* seedlings occurred near pH 6.6 for soil pH values ranging from 4.9 to 7.1, while greatest nodulation of *A. glutinosa* occurred at pH 4.9 over the same pH range. Nodulation was not affected by total soil nitrogen concentrations ranging from 0.09 to 0.20%. Mollisol pH was significantly lower under *A. glutinosa* trees than under *E. angustifolia* trees. For 4- to 8-year-old field-grown trees, *A. glutinosa* nodule weights were negatively correlated with soil pH, while for similar aged *E. angustifolia* trees nodulation in the acidic Alfisol was not detected.

Introduction

There are many genetic and environmental factors that affect actinorhizal plant infection and nodulation by *Frankia*. Genetic variation among *Frankia* isolates is large (Normand and Lalonde, 1986) and host specificity is not always uniform within an actinorhizal plant genus (Weber et al., 1987). Persistence of *Frankia* in soil may depend on the presence of a host plant (Wollum et al., 1968) or the production of spores (van Dijk, 1984). However, some *Frankia* strains seem to be successful soil saprohytes when soil pH and nutrient levels are similar to those optimal under in vitro conditions (Burggraaf et al., 1981; Smolander et al., 1988). *Frankia* have been found in soils devoid of host plant species (Huss-Danell and Frej, 1986) and *Frankia* infective capacity of soil from beneath non-actinorhizal *Betula pendula* was found to be equal to that of soil from beneath an adjacent stand of actinorhizal *Alnus incana* (Smolander, 1990) which is in the same family, Betulaceae. Nodulation of various host species is differentially affected by soil pH (Quispel, 1954), available nitrogen (MacConnell and Bond, 1957), temperature (Wollum and Youngberg, 1969), moisture availability (Righetti et al., 1986) and interaction with

other soil organisms (Knowlton et al., 1980). Host plant nodulation can differ with *Frankia* strain (Dawson and Sun, 1981), morphological state or age of the strain (Weber et al., 1987) and inoculum density (Houwers and Akkermans, 1981).

Species of actinorhizal Elaeagnus and Alnus have been studied for their potential use as nurse crops for commercially valuable trees (Dawson, 1986; Funk et al., 1979; Van Sambeek et al., 1985). The objective of our experiments was to examine the effects of soil order and host species presence on soil capacities to nodulate two ecologically distinct species of these actinorhizal genera. Nodulation patterns of seedlings in pots filled with field soil and 4- to 8-year-old plants of the same species growing in the same soils under field conditions were described and compared.

Methods

Two experimental plantations of *Juglans nigra* L. (black walnut) interplanted with actinorhizal *Alnus glutinosa* (L.) Gaertn. (black alder) and *Elaeagnus angustifolia* l. (Russian olive) were established in 1978 by the USDA Forest Service North Central Forest Experiment Station in cooperation with the Macon County Conservation District in Illinois, USA (Van Sambeek et al., 1985). Plantations with trees at 3×3 m spacing were located on a bottomland Mollisol and an upland Alfisol in east central Illinois (89°09′W, 39°50′N). The upland Alfisol belongs to the Birkbeck soil series and is a fine silty mixed mesic Typic Hapludalf (Champaign County Soil Survey, 1982). The A horizon was about 30 cm thick with a silt loam texture. The B horizon had a pedogenic accumulation of clay with a clay loam texture to a depth of 120 cm. The mean pH and total nitrogen concentration (Kjeldahl) of the top 15 cm were 5.4 ± 0.2 and $0.12 \pm 0.01\%$, respectively. The bottomland Mollisol belongs to the Lawson soil series and is a fine silty mixed mesic Cumulic Hapludoll (Carroll County Soil Survey, 1975). The A horizon had a silt loam texture and was 140 cm thick overlying a massive alkaline silt loam C horizon. The mean pH and total nitrogen concentration of the top 15 cm were 6.8 ± 0.1 and $0.14 \pm 0.01\%$, respectively. Neither plantation had any recorded history of native or exotic actinorhizal species nor were any actinorhizal species found growing within 0.5 km of either plantation.

Surface soil samples (0–15 cm depth), were collected in December beneath three 8-year-old *A. glutinosa* and three 8-year-old *E. angustifolia* trees from each soil order. Ten soil samples per tree were collected at least 50 cm from the boles of the trees to avoid inclusion of nursery soil in the samples. The soil samples were bulked by tree and stored moist in sealed containers for about four weeks prior to use in the pot study. The experimental design consisted of two soil orders from the root zone of two actinorhizal species and planting with seeds of the same two actinorhizal species for a total of 8 treatments. For each treatment, 42 plastic pots (previously rinsed with 1% sodium hypochlorite solution) were filled with about 700 g moist-weight of fresh field soil, the gravimetric equivalent of 500 g oven-dried soil. Each pot was sown with 20 *E. angustifolia* or 50 *A. glutinosa* seeds in January. Seed had been collected the previous fall from trees of both species on both upland and bottomland sites and stored air-dried at 4°C for two months prior to planting. Seeds were surface sterilized in 20% H_2SO_4 for five minutes prior to planting. Pots with sterile peat were planted as checks for stray *Frankia* propagules in the greenhouse. The 10 cm diameter by 8.5 cm deep pots were kept in a greenhouse from January to June in Urbana, Illinois under natural light with a $22° \pm 3$°C day temperature and a $16° \pm 2$°C night temperature. They were randomly arranged on the greenhouse bench and spaced 25 cm apart to prevent between-pot contamination during watering. From June until harvest in October the plant were kept outside under ambient conditions, arranged as in the greenhouse. During the 9 month growing period all pots received only tap water (pH 7.0) or rain. At the time of harvest the number of seedlings per pot and the number of nodules per seedling were counted. Plant heights at the time of harvest ranged from 0.5 to 1.0 m. Soil pH was measured in a 1 : 1 by weight soil : distilled water slurry. Total soil nitrogen was determined by Kjeldahl digestion (Nelson and Sommers, 1973).

Nodules were collected from 4- to 8-year-old field-grown *A. glutinosa* and *E. angustifolia* trees by excavating 0.25 to 0.5 m^3 of soil from beneath the canopies of 42 trees of each species on each soil order. The excavations were to a depth of 15 cm and not closer than 25 cm to the bole of the tree. Nodules were collected at about two-week intervals during the 250 day growing season. On the first field sampling date no nodules were found on three *E. angustifolia* trees from the Alfisol after excavating about two m^3 of soil from beneath each tree. These soil volumes included half the circumference of each tree to a depth of one metre and a distance of one metre from the base of the trees. Because of the lack of nodules and to avoid root injury, no additional root systems of the remaining Alfisol *E. angustifolia* trees were excavated. Field-collected nodules were separated into fresh and senescent tissues, oven dried, weighed, ground with a mortar and pestle and subsampled for total nitrogen determination. Soil samples from the rhizosphere were also collected at each nodule sampling date and soil pH, total nitrogen concentration and moisture content were determined as described above.

Results

There were unequal numbers of seedlings per pot (4 ± 3), but total oven-dried plant biomass per pot was uniform (14.0 ± 3.9 g pot^{-1}), and numbers of nodules per pot were not significantly correlated with numbers of plants ($r = -0.2$), or total plant biomass ($r = 0.03$). None of the seedlings in the check pots were nodulated indicating a lack of contamination by extraneous *Frankia*. Analysis of variance for treatment effects on soil nodulation capacities accounted for 39% of the variation in observed nodulation (Table 1). The main effects of host species, soil order and host vegetation accounted for most of the variation in nodulation. There were significant two-way interactions between host species and soil order and between host species and host vegetation. There was also significant three-way interaction. Nodulation of all *E. angustifolia* seedlings (19.8 nodules/seedling), shown in Table 2, was significantly greater than that for all *A. glutinosa* (4.1 nodules/seedling). Nodulation capacity of all bottomland Mollisol soil samples (15.1 nodules/seedling), was significantly greater than nodulation capacity for all upland Alfisol soils (4.6 nodules/seedling). All soils from beneath *A. glutinosa* trees had a mean of 11.4 nodules/seedling, approximately twice the nodulation capacity of all soils collected beneath *E. angustifolia* trees (6.5 nodules/seedling). Nodulation of *E. angustifolia* in the Mollisol (46.3 nodules/seedling), was about 12 times greater than in the Alfisol (3.9 nodules/seedling). Soil order did not have a significant effect on nodulation for *A. glutinosa* seedlings. Soil from beneath *A. glutinosa* trees had greater nodulation for *E. angustifolia* seedlings (24.1 nodules/seedling), than soils from under *E. angustifolia* trees (12.5 nodules/seedling), and the trend was similar when soil orders were considered separately. The capacity to nodulate *A. glutinosa* seedlings of soils collected beneath *A. glutinosa* trees growing on the Alfisol (6.3 nodules/seedling)

Table 1. Analysis of variance for the effects of host species, soil order and host species vegetation on *Frankia* nodulation of *A. glutinosa* and *E. angustifolia* seedlings

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value	Probability of >F
Host species	1	8975.6299	8975.6299	20.98	0.0001
Soil order	1	6435.7267	6435.7267	15.04	0.0002
Host vegetation	1	2365.0809	2365.0809	5.53	0.0200
Species × soil	1	16183.4149	16183.4149	37.83	0.0001
Soil × vegetation	1	132.1603	132.1603	0.31	0.5792
Species × vegetation	1	2094.3876	2094.3876	4.90	0.0284
Species × soil × vegetation	1	2895.6284	2895.6284	6.77	0.0102
Error	154	65887.1946	427.8389		
Total	161	104969.2236			

Dependent variable: nodules per seedling Model $R^2 = 0.39$.

Table 2. Nodulation (nodules/plant) of *E. angustifolia* and *A. glutinosa* seedlings by upland Alfisol and bottomland Mollisol soils collected from beneath 4- to 8-year-old *E. angustifolia* and *A. glutinosa* trees

Main effects[a]					
Host species*		Soil order*		Host vegetation*	
E. angustifolia	*A. glutinosa*	Mollisol	Alfisol	*E. angustifolia*	*A. glutinosa*
19.8	4.1	15.1	4.6	6.5	11.4
Two-way interactions[b]					
Host species	*E. angustifolia*			*A. glutinosa*	
Soil order	Mollisol	Alfisol		Mollisol	Alfisol
	46.3 a	3.9 b		3.2 b	5.2 b
Host species	*E. angustifolia*			*A. glutinosa*	
Host vegetation	*E. angustifolia*	*A. glutinosa*		*E. angustifolia*	*A. glutinosa*
	12.5 ab	24.1 a		3.5 b	4.6 b
Three-way interactions[b]					
Host species	*E. angustifolia*			*E. angustifolia*	
Soil order	*Mollisol*			*Alfisol*	
Host vegetation	*E. angustifolia*	*A. glutinosa*		*E. angustifolia*	*A. glutinosa*
	26.7 b	61.0 a		1.9 c	4.9 c
Host species	*A. glutinosa*			*A. glutinosa*	
Soil order	*Mollisol*			*Alfisol*	
Host vegetation	*E. angustifolia*	*A. glutinosa*		*E. angustifolia*	*A. glutinosa*
	4.2 ab	2.0 ab		1.6 b	6.3 a

[a] Paired comparisons followed by * are significantly different at the alpha = .05 level.
[b] Means followed by the same letter in the same row are not significantly different at the alpha = .05 level.

was significantly greater than that for soils from under *E. angustifolia* trees (1.6 nodules/seedling). In contrast, on the Mollisol the influence of *A. glutinosa* trees on soil nodulation of *A. glutinosa* (2.0 nodules/seedling), was not significantly different than that for soils from beneath *E. angustifolia* trees (4.2 nodules/seedling).

The relationship of seedling nodulation with soil pH shown in Figure 1 suggests that greatest nodulation in these soils occurs near pH 6.6 for *E. angustifolia* and near or below pH 4.9 for *A. glutinosa*. Nodulation did not seem to vary with nitrogen concentrations (0.09–0.20%) found in the soils of our study.

Despite the apparent lack of nodules, survival and growth of *E. angustifolia* trees were greater on the Alfisol than on the Mollisol (Van Sambeek et al., 1985), perhaps due to wet soil conditions including winter and spring flooding of the Mollisol plantation by the Sangamon river. Mean single nodule dry weight for *A. glutinosa* trees on the Alfisol (0.47 g) was significantly greater than that for *A. glutinosa* trees on the Mollisol (0.27 g), and both were significantly greater than for *E. angustifolia* trees on the Mollisol (0.01 g), (Table 3). Field-collected *A. glutinosa* nodules had mean percentages fresh tissue of 96.7% for the Mollisol and 97.7% for the Alfisol which were not significantly different. Most *E. angustifolia* nodules had some portion that was necrotic resulting in a mean of 33.4% fresh tissue. Despite having a significantly lower percentage fresh tissue than *A. glutinosa*, the smaller *E. angustifolia* nodules had a significantly greater mean total nitrogen concentration (2.90%), than the Mollisol and Alfisol *A. glutinosa* nodules (1.54% and 1.48% respectively). Soil pH of the top 15-cm of the Mollisol under *A. glutinosa* trees (6.7), was significantly lower than under *E. angustifolia* trees (6.9). Soil pH of the Alfisol under *A. glutinosa* trees (5.4), was significantly lower than either Mollisol pH. Total soil nitrogen of the top 15 cm of the Mollisol under *E. angustifolia* tress (0.14%), and under *A. glutinosa* trees (0.13%) did not differ and neither pH differed from that under *A. glutinosa* trees on the Alfisol (0.12%). *A. glutinosa* nodule weights were negatively correlated with soil pH ($r = -0.25$). For *E. angustifolia* trees, nodule total nitrogen concentration was correlated with

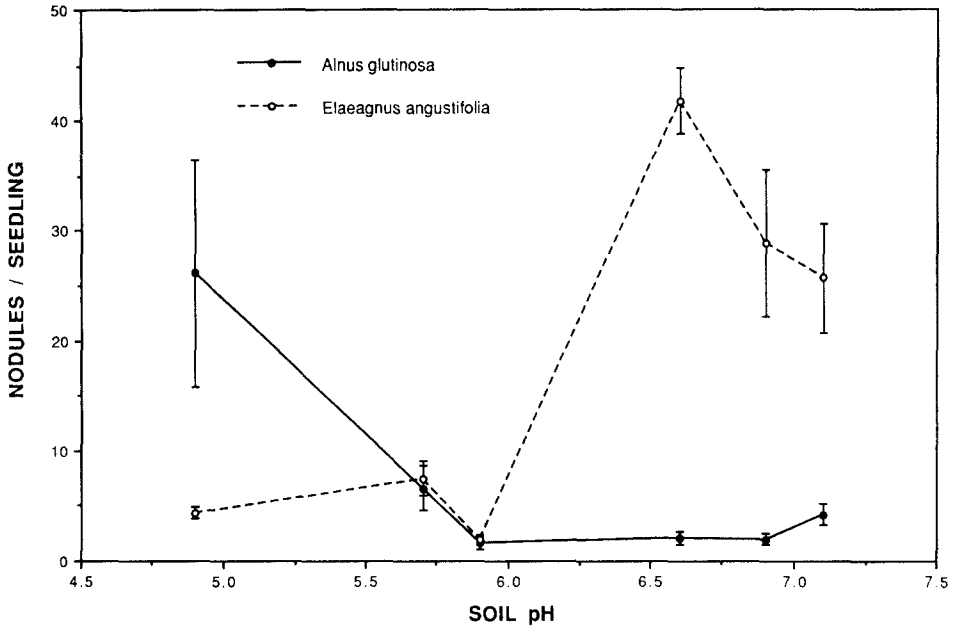

Fig. 1. Relationship of nodulation of *E. angustifolia* and *A. glutinosa* seedlings with pH of field soil. Error *bars* equal ± standard error.

Table 3. Comparison of nodule characteristics of 4- to 8-year-old *A. glutinosa* and *E. angustifolia* and soil pH and total nitrogen from an upland Alfisol and a bottomland Mollisol

	Nodule dry weight (g)	% Fresh tissue	Nodule % total N	Soil pH	Soil % total N
Mollisol *E. angustifolia*					
Mean[a]	0.098 c	33.4 b	2.90 a	6.9 a	0.14 a
Std. dev.	0.086	19.1	0.30	0.3	0.03
Mollisol *A. glutinosa*					
Mean	0.271 b	96.7 a	1.54 b	6.7 b	0.13 a
Std. dev.	0.192	6.8	0.24	0.2	0.02
Alfisol *A. glutinosa*					
Mean	0.470 a	97.7 a	1.48 b	5.4 c	0.12 a
Std. dev.	0.347	5.2	0.25	0.2	0.01

[a] Means followed by the same letter in the same column are not significantly different at the alpha = 0.05 level, n = 210 for nodules and n = 42 for soils.

soil pH (r = 0.64), while nodule percentage fresh tissue was negatively correlated with soil moisture (r = −0.36).

Discussion

Nodules were found on 4- to 8-year-old trees growing in soil with no recent history of host species presence. The 4- to 8- year presence of *A. glutinosa* trees seemed to enhance overall soil nodulation capacity compared to the presence of *E. angustifolia* trees. The apparent host vegetation effects on indigenous soil *Frankia* capable of nodulating *A. glutinosa* trees differed between soils, but the pattern did not differ between soils, for *E. angustifolia* trees.

No record of actinorhizal plants in either soil exists, though both soils are within the natural range of actinorhizal *Ceanothus americanus*

Gleason and Cronguist, 1963). Actinorhizal *Alnus incana* ssp. *rugosa*, *Shepherdia canadensis* and *Comptonia peregrina* occur naturally about 150 km to the north and *Alnus serrulata* occurs naturally about 200 km to the south of the study area (Petrides, 1972). The upland Alfisol, which formed under hardwood forest vegetation (Champaign County Soil Survey, 1982), had supported a brome grass-clover (*Bromus* sp.-*Trifolium* sp.) pasture for about 10 years prior to the planting of actinorhizal plants. During the previous 100 years, there had been significant erosion of the Alfisol surface soil due to row-crop production. Mechanisms of *Frankia* migration are poorly understood (Van Dijk, 1979), but relatively rapid movement of infective *Frankia* into recently deglaciated areas has been observed (Lawrence et al., 1967). Dormant spores, continuous saprophytic growth of *Frankia* under mildly acidic conditions or periodic immigration could have been responsible for maintaining the *Frankia* nodulation capacity of the upland Alfisol.

The Mollisol was located on the flood plain of the Sangamon river and from mid November to late December this soil was under one to two meters of flood water. There is usually an annual net deposition of sediments on this floodplain from winter and spring floods. Deposition of sediments on the Mollisol from the entire upstream watershed include organic matter and nutrients and possibly *Frankia* propagules. Annual flooding and near neutral pH for the Mollisol may also result in a better environment for saprophytic survival of *Frankia* and a greater *Frankia* immigration potential compared to the upland Alfisol. This may explain the three-fold greater nodulation capacity for the Mollisol compared with the Alfisol, regardless of host species.

The relationship between nodulation capacity and soil pH was positive for *E. angustifolia*, but was negative for *A. glutinosa*. These results suggest differential inhibition of host specific *Frankia* populations and/or differential regulation of host infection processes. Nodulation of *A. glutinosa* seedlings has been positively correlated with soil pH when the range was from 3.4 to 5.7 (Smolander, 1990), but not when the pH ranged from 6 to 7 (Dawson and Klemp, 1987) which is consistent with the results of this study.

The negative correlation between field-grown nodule percentage fresh tissue of *E. angustifolia* and soil moisture suggests that insufficient soil aeration existed in some instances for optimal growth of *E. angustifolia* nodules. Furthermore, poor nodulation and greater soil aeration were associated with in greater growth and survival of *E. angustifolia* on the Alfisol compared to the Mollisol.

Nitrogen levels of the top 15-cm of soil were not significantly different between soils nor between host species influence in the same soil. However, the A1 horizon of the Mollisol was almost 1.5 m thick whereas for the Alfisol it was only about 15 cm thick so that total nitrogen in the rooting zone was estimated to be 10 times greater for the Mollisol. Infection processes and subsequent nodule growth are not regulated by the same edaphic factors (Quispel, 1954). Thus, the significantly larger individual nodule mean weights for *A. glutinosa* on the Alfisol compared to *A. glutinosa* on the Mollisol may have been a response to a more limited pool of available soil nitrogen in the Alfisol. This conclusion was also supported by greater nitrogenase activities, estimated by acetylene reduction rates (μmoles C_2H_4/gram dry nodule hour), by field-grown Alfisol *A. glutinosa* nodules compared to Mollisol *A. glutinosa* nodules (Zitzer and Dawson, 1989).

Frankia isolates that nodulate *E. angustifolia* are usually incapable of nodulating *A. glutinosa* (Baker, 1987). *Frankia* isolates from *C. americanus*, *Myrica gale*, *C. peregrina* and *Elaeagnus commutata* were infective with *E. angustifolia* while isolates from *A. incana* ssp. *rugosa*, *Alnus viridis* ssp. *crispa* and *C. peregrina* nodulated *A. glutinosa*. Several of these host species are native to areas within 200 km of this study and could be providing a continuous supply of *Frankia* via wind, water or biotic transport. However, there is some evidence in this study that nodule decay or turnover may be increasing *Frankia* in both soils. It has been proposed that 20-year-old *A. glutinosa* maintain a continuous supply of infective *Frankia* in the soil via nodule decay (van Dijk, 1978). Thus, greater *A. glutinosa* nodulation capacity of the Alfisol from beneath *A. glutinosa* trees compared with that of the Alfisol from beneath *E.*

angustifolia trees may have been due to nodule turnover. However, high apparent nodule turnover for *E. angustifolia* on the Mollisol did not result in greater *E. angustifolia* seedling nodulation capacity compared to the Mollisol from beneath *A. glutinosa* trees. Thus, actinorhizal plants can apparently increase soil infective capacities irrespective of *Frankia* host specificity.

In conclusion, we found significant differences in nodulation capacities of soils for *A. glutinosa* and *E. angustifolia* seedlings at a locale in east central Illinois. Differences were associated with soil order, soil pH, host species presence in the field and the host seedling species planted. Apparently considerable *Frankia* diversity existed in these central Illinois soils as evidenced by their ability to promote nodulation of exotic hosts from different plant families.

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