Influence of Nonsymbiotic and Symbiotic Parameters of Underground Organs on the Development of Aboveground Organs in *Pinus sylvestris* Seedlings

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Abstract—A method is described for comparing the influences of nonsymbiotic and symbiotic parameters (accounted for by the plant itself and by the plant together with ectomycorrhizal fungi, respectively) of underground organs on the development of aboveground organs. The method has been used to analyze 4- to 12-month Scots pine seedlings from 26 habitats. The results show that 24 to 88% (on average, 54%) of total variation in the weight of aboveground organs is dependent on specific structural features of roots and mycorrhizae, with nonsymbiotic and symbiotic parameters of underground organs accounting for 5–73% (on average, 36%) and 3–45% (on average, 18%) of this variation.

Keywords: ectomycorrhizal symbiosis, significance of symbiosis, multiple regression, Scots pine.

Evidence for the positive significance of mycotrophism for tree growth and productivity has been obtained mainly in laboratory experiments (Shemakhanova, 1962; Cudlín et al., 1983; Sudhakara and Natarajan, 1997; Wallander, 2000; Ahonen-Jonnarth and Finlay, 2001; Niemi et al., 2005; Chen et al., 2006) and, to a much lesser extent, in field studies (Marx and Bryan, 1975; Valdes, 1986; Khasa et al., 2001; etc.). Although ectomycorrhiza formation is sometimes accompanied by a decrease in the biomass of the plant symbiont (Colpaert et al., 1992; Roldán and Albaladejo, 1994; Wallander et al., 1997; Heijden and Kuyper, 2001; Rincon et al., 2001), the biomass of seedlings infected by ectomycorrhizal fungi is usually 10–50% greater than that of nonmycorrhizal seedlings. The contribution of ectomycorrhizal fungi to plant production at individual level has not been evaluated, since no methods adequate to this task have been developed to date. The method described in this paper allows quantitative comparison between contributions of nonsymbiotic and symbiotic parameters of underground organs to variation in the development of aboveground organs in seedlings of the Scots pine (*Pinus sylvestris* L.), thereby providing the possibility of estimating the functional significance of plant mycotrophism in situ.

In experiments on artificial mycorrhization, the effect of ectomycorrhizal fungi on the state of plants is estimated by comparing parameters of inoculated and control (nonmycorrhizal) individuals. In complex multifactor experiments (e.g., see Heijden and Kuyper, 2001), this principle is strictly observed, and statistical methods for discrete comparisons are sufficient for evaluating the effect of inoculation. In nature, where no nonmycorrhizal control is available, such a method of estimating the significance of ectomycorrhizal symbiosis for plants is inapplicable. A distinctive feature of the approach described below is that the bilateral functional relationship between the aboveground and underground plant organs is reduced to a unilateral influence, with the state of the former being regarded as a function of the state of the latter. With regard to continuity of variation in the parameters of plant state, the type and strength of their influence on each other is estimated by means of multiple regression analysis. Similar approaches have been used previously to assess the significance of ectomycorrhizae for plant growth, including calculation of correlations between corresponding parameters (Brunner, 1987; Last et al., 1989; Berman and Bledsoe, 1998) and individual assessment of morphological characteristics of plants with different levels of ectomycorrhiza development (Shubin, 1965; Lobanov, 1971; Semenova, 1985).

MATERIAL AND METHODS

The proposed approach was verified on 26 samples of 4- to 12-month Scots pine seedlings from pine forests, burned-out areas, natural open habitats, and nurseries (Table 1). The plants were dug out carefully so as to avoid damage to underground organs. Seed-

Table 1. Characteristics of test samples of *Pinus sylvestris* seedlings

Sample no.	Geographic location		Habitat*	Seedling age	Sample size, ind.	Structural parameters of seedlings ($m \pm SE$)		
	N	E				Aboveground phytomass, mg	Conducting root length, mm	Mycorrhiza-tion rate, %
1	55°36′	65°02′	Forest 1	12	30	18 ± 1	97 ± 7	84 ± 4
2	55°36′	65°02′	"	12	37	35 ± 4	181 ± 15	86 ± 1
3	55°36′	65°02′	"	12	65	32 ± 2	195 ± 14	86 ± 2
4	55°36′	65°02′	Forest 2	12	33	26 ± 3	171 ± 14	53 ± 6
5	55°36′	65°02′	"	12	32	22 ± 3	153 ± 13	43 ± 5
6	55°36′	65°02′	Burned-out area 1	12	45	30 ± 3	219 ± 15	63 ± 4
7	55°36′	65°02′	"	12	44	38 ± 4	253 ± 15	55 ± 4
8	55°36′	65°02′	"	12	46	40 ± 4	226 ± 14	59 ± 4
9	55°36′	65°02′	Burned-out area 2	12	30	32 ± 3	226 ± 19	63 ± 4
10	55°36′	65°02′	"	12	30	43 ± 5	248 ± 22	61 ± 4
11	56°57′	60°21′	Forest 3	5	60	11 ± 0	109 ± 5	76 ± 2
12	56°57′	60°21′	"	5	31	12 ± 1	152 ± 10	78 ± 4
13	56°55′	60°34′	Clay pit	5	30	29 ± 2	516 ± 31	34 ± 4
14	56°55′	60°34′	"	5	32	20 ± 1	400 ± 19	33 ± 4
15	56°55′	60°34′	"	5	30	22 ± 2	510 ± 40	21 ± 3
16	57°01′	60°18′	Peat deposit	5	30	49 ± 2	423 ± 21	6 ± 1
17	57°01′	60°18′	"	5	30	52 ± 2	590 ± 28	6 ± 1
18	57°01′	60°18′	"	5	31	46 ± 3	612 ± 38	6 ± 2
19	56°57′	60°46′	Nursery 1	6	39	50 ± 5	440 ± 30	11 ± 1
20	56°41′	60°32′	Nursery 2	6	32	124 ± 11	752 ± 49	6 ± 1
21	56°25′	60°34′	Nursery 3	4	65	129 ± 11	525 ± 24	9 ± 1
22	56°25′	60°18′	Nursery 4	6	30	39 ± 3	336 ± 23	32 ± 2
23	56°46′	59°58′	Nursery 5	6	50	43 ± 4	244 ± 13	28 ± 2
24	55°36′	65°02′	Nursery 6	6	40	92 ± 9	501 ± 34	12 ± 2
25	56°29′	60°47′	Nursery 7	6	40	35 ± 2	333 ± 16	43 ± 1
26	56°57′	59°55′	Nursery 8	6	40	45 ± 4	477 ± 21	28 ± 2

* Forest 1, cowberry−herb−moss pine forest; forest 2, the same forest after creeping fire (burned-out area with sparse tree stand); burned-out area 1, the same forest after total fire (burned-out area with dead standing trees); burned-out area 2, the same with tree stand cut down (burned-our cutover area); forest 3, dead-cover pine forest on cliffs; clay pit, slopes of a clay pit; peat deposit, drained lowland peat bog; nurseries 1−8 are located in (1) Berezovskii, (2) Sverdlovskii, (3) Sysertskii interregional, (4) Polevskii, (5) Revdinskii, (7) Sysertskii, and (8) Bilimbaevskii forest enterprises of Sverdlovsk oblast and (6) Prosvetskii forest enterprise of Kurgan oblast.

lings with damaged root systems were sorted out in the laboratory to be excluded from analysis.

Structural parameters of seedlings were studied after fixation in 4% formaldehyde solution. The weight (mg) of the aboveground plant part (above the root collar) was determined after drying at 105°C for 24 h. Other test parameters were as follows: (1, 2) the lengths (mm) of the main root and of undetermined first-order lateral roots, all referred to as conducting roots; (3) the total length (mm) of conducting roots; (4−6) the numbers of nonmycorrhizal absorbing roots, mycorrhizae, and mycorrhizal endings; (7) the rate of root system mycorrhization, or the percent ratio of the number of mycorrhizae to the number of absorbing roots; and (8−10) the densities of nonmycorrhizal roots, mycorrhizae, and mycorrhizal endings determined as the numbers of corresponding structures per 100 mm of conducting root length (Selivanov, 1981). The term "nonmycorrhizal absorbing root" refers here to a determined last-order lateral root uninfected by ectomycorrhizal fungi; the term "mycorrhiza," to the organ derived from a determined lateral root as a result of its colonization by an ectomycorrhizal fungus; and the term "mycorrhizal ending," to an individual branch of a complex mycorrhiza.

Table 2. Average parameters of equations describing the dependence of shoot weight in *Pinus sylvestris* seedlings on three principal factors (PF) characterizing the development of mycorrhizae ($beta_1$), conducting roots ($beta_2$), and nonmycorrhizal absorbing roots ($beta_3$)

Group no.	Criteria of group formation	Number of habitats	Regression coefficients			Proportions of explained variance		
			$beta_1$	$beta_2$	$beta_3$	SS_s	SS_{ns}	SS_u
0	All cases	26	+0.36	+0.54	+0.09	0.18	0.36	0.54
1	Both $beta_1$ and $beta_2$ values are significant, including	17	+0.48	+0.53	+0.06	0.24	0.34	0.58
1a	$beta_1 > beta_2$	7	+0.57	+0.34	+0.01	0.36	0.20	0.56
1b	$beta_1 < beta_2$	10	+0.42	+0.65	+0.09	0.15	0.44	0.59
2	Both $beta_2$ and $beta_3$ values are significant	3	+0.07	+0.52	+0.43	0.04	0.54	0.58
3	Only $beta_2$ values are significant	6	+0.16	+0.56	−0.01	0.07	0.35	0.42

Nonmycorrhizal and mycorrhizal roots (ectomycorrhizae) were identified under a binocular microscope at 10−20× magnification by their branching pattern, shape, and the presence of surface hyphal structures. All test parameters were divided into two groups, symbiotic (s) and nonsymbiotic (ns), by the criterion of involvement of ectomycorrhizal fungi in their formation. Thus, parameters 1−4 and 8 were classified as nonsymbiotic, and parameters 5−7, 9, and 10, as symbiotic.

A major requirement for the set of predictors in multiple regression analysis is that they should be independent from each other. The above set of test parameters does not satisfy this requirement, since many of them correlate with each other. Moreover, the number of potential predictors (ten parameters) is excessive for constructing regression models on the basis of 30−60 observations. To reduce the number of predictors, factor analysis was used (Veselkin, 2008), which showed that all test parameters consistently clustered into three readily interpretable groups of principal factors (PFs): $PF1_s$, symbiotic parameters characterizing the development of ectomycorrhizae; $PF2_{ns}$, parameters describing the development of conducting roots; and $PF3_{ns}$, the number of nonmycorrhizal absorbing roots. The key parameters of underground organs weakly correlating with each other and generally connected with only one PF were as follows: the density of mycorrhizal endings ($PF1_s$), the total length of conducting roots ($PF2_{ns}$), and the density of nonmycorrhizal roots ($PF3_{ns}$).

RESULTS AND DISCUSSION

The dependence of the aboveground phytomass of pine seedlings on the degree of development of their underground organs is described by the following equation:

$$m' = beta_1 x_1' + beta_2 x_2' + beta_3 x_3',$$

where m' is the standardized value of the aboveground phytomass; $beta_1$, $beta_2$, and $beta_3$ are standardized regression coefficients; and x_1', x_2', and x_3' are standardized PF estimates describing the development of ectomycorrhizae (x_1), conducting roots (x_2), and nonmycorrhizal absorbing roots (x_3).

In all habitats, the aboveground phytomass (shoot weight) of seedlings positively correlated with the development of conducting roots (Table 2). A significant effect of symbiotic parameters on shoot weight was revealed in 17 habitats (65%); in seven habitats, shoot weight depended more strongly on the development of ectomycorrhizae rather than on the development of conducting roots. A significant effect of the variable describing the development of nonmycorrhizal absorbing roots was observed in three habitats (12%). There were no cases where the variables describing the development of symbiotic and nonsymbiotic absorbing roots were found to have a significant simultaneous influence on the shoot weight.

The values of *beta* coefficients in 26 samples averaged 0.36 ($beta_1$), 0.54 ($beta_2$), and 0.09 ($beta_3$), with medians of their distributions being 0.42, 0.52, and 0.06, respectively. Therefore, the aboveground shoot growth in all these habitats depended mostly on the development of conducting roots (characterized by the $beta_2$ value). However, total averaging of regression coefficients is poorly informative, since their values are highly variable. It was possible to distinguish three types (groups) of combinations of statistically significant regression coefficients (Table 2), the analysis of which has shown that the effect on shoot weight of variables characterizing the development of ectomycorrhizae or nonmycorrhizal roots is sometimes comparable to that of the variable characterizing the development of conducting roots.

Undetermined (conducting and skeletal) roots and determined (nonmycorrhizal absorbing and mycor-
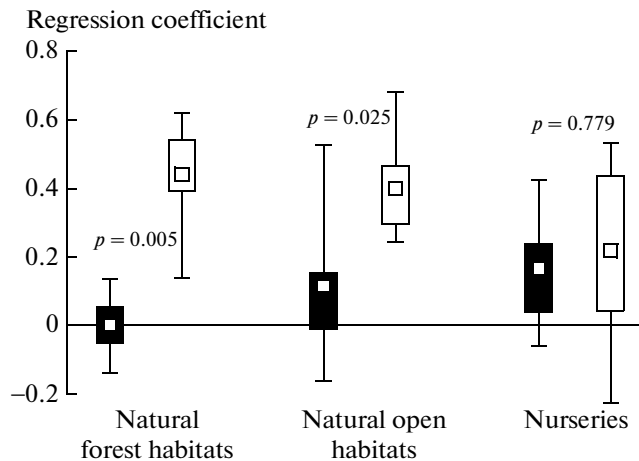
Regression coefficient

Fig. 1. Values of regression coefficients characterizing the dependence of shoot weight in *Pinus sylvestris* seedlings on the development of nonmycorrhizal absorbing roots ($beta_3$, solid bars) and ectomycorrhizae ($beta_1$, clear bars) in different habitats.

Here and in Fig. 2, squares show mean values; bars, interquartile ranges; vertical lines, minimum and maximum values. Significance of differences between $beta_1$ and $beta_3$ values in each group of habitats is indicated according to Wilcoxon test for pairwise comparisons.

Proportion of variance

Fig. 2. Components of variance in shoot weight accounted for by nonsymbiotic (SS_{ns}, solid bars) and symbiotic (SS_s, clear bars) parameters of underground organs of *Pinus sylvestris* seedlings in different habitats. Significance of differences between SS_{ns} and SS_s values in each group of habitats is indicated according to Wilcoxon test for pairwise comparisons.

rhizal) roots differ in their basic functions. The development of the former is aimed at expansion and colonization of soil space, whereas the latter provide for the maximum possible utilization of resources contained in the immediate soil environment. With regard to this factor, the effects of symbiotic and nonsymbiotic absorbing roots on shoot growth were compared separately from the effect of conducting roots (Fig. 1). The results show that in natural forest and open habitats it is expedient for pine seedlings to gain increasing numbers of ectomycorrhizae, with changes in the number or density of nonmycorrhizal roots having almost no effect. In nurseries, the average effects of increase in the relative contents of symbiotic or nonsymbiotic absorbing organs in the root system are comparable.

Contributions of nonsymbiotic and symbiotic parameters to variation in shoot weight were characterized via the proportions of variance in the aboveground phytomass accounted for by nonsymbiotic (SS_{ns}) and symbiotic (SS_s) parameters of underground organs:

$$SS_u = SS_{ns} + SS_s,$$

where SS_u is the total variance in the aboveground phytomass of pine seedlings accounted for by structural features of their underground organs.

In different habitats, these features accounted for 24 to 88% of the total variance in shoot weight; the interquartile range of SS_u values was 45−70% with a median of 53%. Nonsymbiotic parameters accounted for 5 to 73% of the total variance, the interquartile
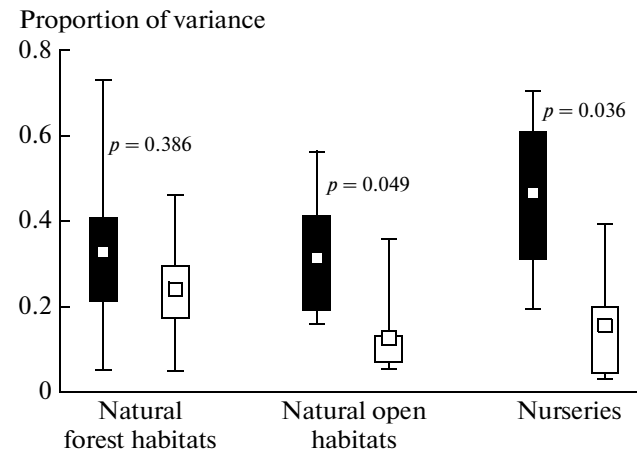
range was 20−51% with a median of 33%; for symbiotic parameters, the respective values were 3−45, 7−27, and 15%. The smallest difference between values characterizing the significance of symbiotic and nonsymbiotic parameters was observed in forest habitats (Fig. 2), where the roots of seedlings interact with the complexes of ectomycorrhizal fungi that are sustainably functioning in all soil loci. When Scots pine seedlings are establishing themselves in areas where such complexes are absent (in natural open habitats or nurseries), success in their growth and total variation in shoot weight depend mainly on nonsymbiotic parameters of underground organs.

Changes in underground organs accompanied by an improvement in the development of aboveground organs are regarded as modificational adaptations. These adaptive responses may be accomplished in different ways: first, by plants themselves, via an increase in the length of conducting roots and in the number of nonmycorrhizal absorbing roots; second, via transformation of nonmycorrhizal roots into ectomycorrhizae and subsequent branching of the latter. The first, autonomous way alone proved to be realized in 9 out of 26 habitats studied; in all other habitats, the aboveground phytomass of pine seedlings proved to depend both on the length of the main and lateral undetermined shoots and on success in ectomycorrhiza formation. Attention should be paid to correspondence (although not absolute) between the relative abundance of symbiotic and nonsymbiotic absorbing organs in the root system of a seedling and their significance for its development. The proportion of absorbing roots transformed into ectomycorrhizae in seedlings from nurseries averaged 20% (ranging from 5 to

43%), compared to 30–40% (6–63%) in natural open habitats and 70% (43–86%) in forests. Thus, an increase or decrease in the ratio of nonmycorrhizal roots and ectomycorrhizae is accompanied by a similar change in the strength of their effect on shoot growth, but, on the other hand, this effect of ectomycorrhizae is usually stronger than that of nonmycorrhizal roots even at a low level of root system mycorrhization. Apparently, this trend indicates that the symbiotic strategy of soil nutrition is advantageous for pine seedlings in the greater part of habitats included in the study.

CONCLUSIONS

(1) The proposed approach to differential assessment of the effects of different structural components of the root system on the development of aboveground plant organs is feasible. It allows quantitative comparison between contributions of symbiotic and nonsymbiotic components to the development of ectomycorrhizal plants in the absence of "pure" nonmycorrhizal control, i.e., under conditions of natural biogeocenoses.

(2) The set and rank of root system parameters significant for the development of plants widely varies depending on habitat conditions. The data presented above indicate that the significance of mycotrophism for *P. sylvestris* seedlings increases upon transition from incomplete pioneering or cultivated communities (biogeocenoses) to well-developed, relatively complete natural communities (biogeocenoses).

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