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# Use of soil transfer for reforestation on abandoned mined lands in Alaska

## I. Effects of soil transfer and phosphorus on growth and mycorrhizal formation by *Populus balsamifera*

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**Abstract.** Soil transfers, presumed to contain mycorrhizal fungal inoculum from a native plant community, were applied to rooted cuttings and volunteer plants of *Populus balsamifera* L. to improve their growth on an abandoned mined site in south central Alaska. The objectives of the study were to determine (1) if these soil transfers could be substituted for additional P fertilizer when applied with a base level of NP fertilizer to improve growth, and (2) if P added to treatments receiving a base level of NP fertilizer and soil transfer would suppress mycorrhizal formation. Physical dimensions of plants were measured near the beginning and end of each of two growing seasons. Several plants per plot were harvested at the end of year 2 to determine mycorrhizal formation, current growth, and leaf nutrient concentrations. Plant height was significantly increased only when soil transfer and additional P treatments were combined. Response to additions of P fertilization alone or soil transfer alone were not significantly different from each other. Current twig growth increased with either treatment alone or both combined. Soil transfer on cuttings resulted in more ectomycorrhizal formation than either the control or additional P. Leaf N concentrations on cuttings and volunteers increased when plants were treated with soil transfer. Similar growth responses were achieved by soil transfers or additional P, but mycorrhizal formation was improved with the soil transfer treatments. P added to the base level plus soil transfer did not suppress or improve mycorrhizal formation compared to that with soil transfer alone.

**Key words:** Soil transfer – Reforestation – *Populus* – Phosphorus – Boreal forest

## Introduction

Natural revegetation of abandoned mined lands is a slow process on dry, nutrient-poor sites even when plant propagules are available. Soil stabilization and re-establishment of normal ecosystem processes may take even longer in cold regions, such as the boreal forest zone of Alaska. To facilitate natural succession and to reclaim these drastically disturbed sites that contain no topsoil, low-cost techniques of re-establishing woody species must be developed. Past revegetation projects initially have applied seed and fertilizer to establish good ground cover and stabilize soil. However, these seeded species have not maintained themselves without periodic additions of fertilizer.

A more economical and longer lasting alternative may be to reintroduce mycorrhizal fungi adapted to local natural vegetation onto the sites identified for revegetation (Aldon 1975; Parkinson 1978). Mycorrhizal fungi, as well as other soil microorganisms, are essential to normal ecosystem function, but population levels may be reduced or eliminated during disturbances. Inoculating seedlings with either pure cultures of mycorrhizal fungi or forest soil has been found to increase mycorrhizal formation by seedlings planted on mine spoils, unreforested clearcuts, or sites where the mycorrhizal fungi required by the seedlings do not occur naturally (Bjorkman 1970; Mikola 1973; Marx and Artman 1979; Amaranthus and Perry 1987; Perry et al. 1987; Danielson 1988). Inoculated seedlings generally grow better than uninoculated seedlings, partly because faster root and mycorrhizal development permits seedlings to absorb nutrients and water needed for rapid establishment (Perry et al. 1987; Amaranthus and Perry 1987, 1989). Also fungal hyphae help stabilize soils by improving soil aggregation (Sutton and Sheppard 1976; Koske and Halvorson 1981; Koske and Polson 1984) and protecting against pathogens (Marx 1972).

Natural dispersal and survival of mycorrhizal fungal propagules on disturbed sites may be insufficient to maintain mycorrhizal inoculum density at levels found in undisturbed soils (Perry et al. 1987). However, dispersal of propagules usually is sufficient to maintain at least some inocula even on highly disturbed sites. For example, mycorrhizae have been found on plants

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growing on anthracite and bituminous coal wastes of Pennsylvania (Daft and Hacskaylo 1976), on disturbed alpine sites (Allen et al. 1987), and on a reclaimed stripmine in Wyoming (Allen and Allen 1980). To improve plant growth on low nutrient spoils, adequate quantities of inoculum of appropriate mycorrhizal fungal species should be introduced at the time of planting. Mycorrhizal fungi introduced in this way have led to improved rates of establishment of woody species on mined lands (Aldon 1975; Call and McKell 1982; Williams and Allen 1984) and on old logged sites (Amaranthus and Perry 1987, 1989).

Soil disturbance can reduce populations of mycorrhizal fungi, so nonmycorrhizal plants are favored as colonizers in temperate climates (Reeves et al. 1979; Allen and Allen 1980). However, in Alaska, most colonizing plant species can be mycorrhizal, and in fact the woody pioneers can be either vesicular-arbuscular (VAM) or ectomycorrhizal (ECM). Janos (1980a, b) has proposed that in the tropics, initial colonizers are not dependent on mycorrhizae, intermediate successional species are facultatively dependent and climax species are obligately dependent on mycorrhizae. E. Allen (1984) indicated a similar relationship in Wyoming sagebrush grassland. In order to assist natural succession, M. Allen (1984) has proposed that plant species used for revegetation should promote mycorrhizal inoculum development, but not be highly dependent on mycorrhizal associations. Plant species more dependent on mycorrhizae could become established later after mycorrhizal inoculum density increases.

Formation of mycorrhizae may depend on plant and fungal species as well as on site conditions (Mason et al. 1983), especially nutrients (Hayman 1982; Allen and Allen 1990). Soil fertility must be sufficient for mycorrhizal formation, but not so great as to suppress their formation. Use of local mycorrhizal fungi that occur in established vegetation communities would ensure that the fungi are adapted to the regional climate although not necessarily to the specific environment of the disturbed site.

Several methods may be used to introduce mycorrhizal inoculum onto a site. Cuttings or seedlings may be inoculated in nurseries or greenhouses before planting (Marx and Cordell 1988; Danielson and Visser 1988, 1989; Castellano and Molina 1989). A simple field technique is the "soil-transfer" method, in which soil from adjacent native communities is transferred to the rooting zone of seedlings or cuttings at the time of planting on disturbed sites (Pilz and Perry 1984; Amaranthus and Perry 1987). This soil transfer from feeder-root zones presumably contains climate-adapted mycorrhizal fungi in the form of mycelia, spores, and/or infected plant roots as well as bacteria and other microorganisms. Bacteria may help or hinder mycorrhizal formation (Bowen and Theodorou 1979). Treatment with a combination of bacteria and mycorrhizae may improve plant growth compared with treatment with either organism alone (Azcon et al. 1976). This soil-transfer technique has been used successfully to improve re-establishment of *Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir) on old clearcuts in Oregon (Amaranthus and Perry 1987, 1989).

A series of experiments was designed to determine the suitability of soil transfer to facilitate establishment of *Populus balsamifera* L. (balsam poplar) on abandoned mined lands in south central Alaska. *P. balsamifera* was selected as the target plant species for several reasons. (1) Poorly-developed individuals of *P. balsamifera* were already colonizing the site. (2) *P. balsamifera* individuals can be infected by both VAM and ECM fungi (Vozzo and Hacskaylo 1974; Rothwell and Vogel 1982; Lodge and Wentworth 1990), yet they can grow without mycorrhizae initially. Once *Populus* becomes infected with mycorrhizal fungi, these fungi may spread to other plant species. (3) *Populus* also forms adventitious roots and would not be adversely affected by soil deposition (e.g. slides) that may occur naturally on unstable slopes.

Two general objectives of this series of experiments were (1) to compare results of soil transfers and fertilization and (2) to evaluate different soils as sources for the soil transfers. The first general objective is addressed in this paper while the second is addressed in the following paper (Helm and Carling 1993). Specific objectives of the study reported in this paper were to determine (1) whether soil transfer could be substituted for additional P fertilization and (2) whether the application of this additional P fertilizer would suppress the mycorrhizal fungi associated with the soil transfer. This soil-transfer technique, if successful, potentially can restore one aspect of ecosystem function if the mycorrhizae can maintain themselves on these disturbed sites. This would reduce or eliminate the need for additional amendments over time.

## Materials and methods

### Study area

The study area consisted of overburden on lands mined for coal near Jonesville, Alaska (61° 44′ 148° 56′W). The mine was abandoned during the 1960s. Since abandonment, scattered individuals of young *Populus balsamifera* and *Betula papyrifera* Marsh. (paper birch) have naturally colonized the mine spoil. Although most plants were 5 to 10 years old, most stems were <60 cm tall. Other scattered plant species on the disturbed site included *Equisetum arvense* L., *Elymus sibiricus* L., *Taraxacum officinale* Wiggars, and *Epilobium angustifolium* L. Most ground cover and density was provided by *P. balsamifera.* Study plots were placed in a relatively homogeneous portion of this area. An intermediate-successional vegetation community developed a closed canopy on an older disturbance near the study plots and consisted of 20- to 30-year-old *P. balsamifera* and *Alnus crispa.* Soil transfers were collected from this community.

### Plant material preparation

Dormant *P. balsamifera* cuttings were collected in mid- to late April 1988 from young *P. balsamifera* trees near the study area. Cuttings were stored just above freezing then rooted in a mist bed. Rooted cuttings were transplanted from the mist bed to pots

containing a sterilized mixture of coarse sand and potting soil. Potted plants were set outside for hardening.

## *Preliminary soil and mycorrhizal surveys*

Pre-experiment spoil samples were collected for the mine spoils on each plot on 2 June 1988, and analyzed for extractable N, P, and K to determine fertilizer levels desired for experimental treatments. The sample for each replicate (block) consisted of a composite of 15–25 cores 10 cm deep × 2.5 cm diameter.

Spoil samples were collected, air-dried, and sieved to remove the fraction with particles more than 2 mm in diameter. The fraction of particles less than 2 mm was analyzed for the following parameters: extractable inorganic $NH_4^+$, $NO_3^-$ (2-N KCl, Bremner 1965); total N (digestion in $H_2SO_4$-$H_2SeO_3$, Isaac and Johnson 1976; Bremner and Mulvaney 1982); extractable P (Olsen's, Olsen and Sommers 1982); total P (digestion in $H_2SO_4$-$H_2SeO_3$, Isaac and Johnson 1976); K, Ca, Mg, Na (Mehlich 3, Mehlich 1982; $NH_4OAc$, Pratt 1965); cation exchange capacity ($NH_4OAc$, SCS 1984); organic carbon (Walkley-Black, Allison 1982; Jackson 1958); and pH (1:1 and paste with water, Peech 1965). These analyses were performed by the Plant and Soil Analysis Laboratory of the Agricultural and Forestry Experiment Station, University of Alaska Fairbanks.

Root samples were collected from native and disturbed vegetation types near the study plots to determine the presence of mycorrhizae (Helm and Carling 1990). These samples were returned to the laboratory and examined for ectomycorrhizae and endomycorrhizae. VAM spores were separated from the soils surrounding the roots. Soil transfer remaining after the field treatments were also analyzed for mycorrhizae to confirm their presence in the soil transfer.

## *Plot layout*

A factorial randomized complete block design was used for three treatments with two levels each: (1) plant material (rooted *P. balsamifera* cuttings, *P. balsamifera* volunteers), (2) fertilizer (base level or operational control = 100 mg/kg N + 50 mg/kg P, base level plus additional P = 100 mg/kg N + 100 mg/kg P), and (3) soil transfer (none, soil transfer assumed to contain mycorrhizal fungi). The design was replicated three times with 10 rooted cuttings (30 plants per treatment) or five volunteers (15 plants per treatment) in each cell. Fewer volunteers were used in each treatment because survival of volunteers was expected to be greater than that of the cuttings.

Plots were laid out on a relatively unvegetated, homogeneous portion of the spoils. Plots were sized 2 m × 5 m and were arranged in two columns so that the eight plots within a block covered an 8 m × 10 m area. Treatments were randomly assigned to each plot. Volunteer plants were selected from among the small plants already growing in the plots designated as volunteer plots. Volunteer plants were selected to be similar in height to the rooted cuttings being outplanted. Other volunteers were uprooted or clipped back depending on their size and proximity to experimental plants.

## *Soil transfer and planting*

Soil transfer was collected from the top 5–10 cm of soil in the feeder-root zone of several *P. balsamifera* plants in a relatively homogenous portion of the *P. balsamifera* – *A. crispa* stand. This soil transfer was collected, transported to the experimental site about 50 m away, homogenized by hand, and used the same day.

Spacing between outplanted rooted cuttings was 70 cm, a spacing equal to the mean distance between volunteers on the site. Cuttings were placed in holes sized 20 × 20 × 20 cm on 30 June, 1988. In plots with mycorrhizal treatments, approximately 250 ml of soil transfer from the *P. balsamifera* – *A. crispa* community was placed in the bottom of each planting hole and incorporated around the roots of the cuttings. Some spoil was returned to the hole, while the appropriate fertilizer treatment was mixed with the remaining spoil as the planting hole was filled. A small hole was dug adjacent to the selected volunteers, and the same amount of soil transfer or fertilizer was incorporated around their roots. Each rooted cutting and volunteer was watered with about 250 ml of tap water to improve soil-root contact and to reduce transplant shock.

## *Estimates of plant growth*

Field measurements taken on all living plants at the start of both growing seasons and the end of the first growing season included height and basal diameter of stems. Final field measurements included plant height, length and width of crown, basal stem diameter, twig length, twig diameter, and qualitative estimates of vigor. Height was measured from the ground to the highest living point on the plant. Initial height was defined as the height when the plant material was started in the greenhouse and was used as a covariate in the statistical analysis. Crown length and width were measured parallel to the earth and perpendicular to each other. These two dimensions were combined to calculate crown area using the formula for an ellipse. Crown area is assumed to be related to root spread, which is an important factor in soil stabilization. The crown also reduces the impact of rain on the spoils. Basal stem diameter was measured about 1 cm above the ground using plastic calipers. Twig length and diameter were the dimensions of the terminal or longest twig on the plant and were indicators of second year growth.

Phenological observations were made on 29 April, 13 May, and 29 May, 1989. Plants were categorized as (0) dead, (1) apparently living but no signs of breaking dormancy, (2) buds swelling, (3) buds breaking, (4) leaves expanding, or (5) twigs growing.

Vigor was estimated on a scale of 1 to 5, with plants receiving a rating of 1 having low vigor and little chance of survival. Plants with average vigor were assigned a 3. Vigor estimates were based on greenness, plant height, fullness of crown, and general appearance.

Thirteen cuttings (about four per plot) or three volunteers (one per plot) in each treatment were harvested at the end of the second year. Most individuals were selected randomly. In most plots, this coincidentally provided a range of plant sizes. In some other plots, individual plants were observed that were particularly interesting because they were more vigorous or less vigorous compared to the randomly selected ones. These special plants sometimes were selected in place of or in addition to the randomly chosen plants so that we could investigate the relation between vigor of cuttings and mycorrhizal formation. This selection process was assumed to increase variances for biomass, root dimension, and mycorrhizal infection analyses compared to random sampling, but our objective of relating vigor and growth with mycorrhizal infection outweighed this increase in variance. The remaining plants were left intact for long-term observations on growth and survival.

## *Plant tissue analyses*

Plants with roots and leaves attached were returned to the laboratory. The roots were allowed to hang naturally and depth and width dimensions measured. Roots were separated from the tops and saved for analysis of mycorrhizal formation. Adhering spoil was shaken loose, and spores were separated from this spoil.

All leaves and first and second year twig growth were separated, bagged separately, oven-dried at 60° C, then weighed to determine oven-dried biomass of each component for each plant. Leaf tissues in each plot were composited, then analyzed for concentrations of N, P, K, Ca, and Mg ($H_2SO_4$-$H_2SeO_3$ digestion, Isaac and Johnson 1976). Twig growth from the establishment year included growth initiated in the greenhouse as well as growth achieved after planting in the field.

### *Mycorrhizal analyses*

Spores of fungal species forming vesicular-arbuscular (VA) mycorrhizae were separated by differential centrifugation from a 17.5-g spoil sample collected from the rhizosphere of each harvested plant (Allen et al. 1979). Spores were counted at 40× under a dissecting microscope.

Root samples were analyzed for ECM and VAM formation. Roots were separated from the stem and washed. Ectomycorrhizal infection percentage was determined initially by counting both infected and uninfected root tips at magnification of ×10–20 under a binocular microscope. Three or more root subsamples per plant were grab-sampled from the mass of roots and the percentage of ECM root tips was determined. Most subsamples contained about 100 root tips. Each mycorrhizal root tip was evaluated by color and general morphology and placed into one of the following categories: white wooly, white smooth, tan smooth, dark brown wooly, or black smooth (Zak 1973). The three subsamples were averaged to obtain one estimate of the percent colonization and composition for each individual.

Following this surface examination, roots from each harvested plant were prepared to determine VAM formation and to confirm ECM development (Hartig net). Roots were cleared and stained following the modified methods of Phillips and Hayman (1970) and Kormanik et al. (1980), including bleaching with alkaline $H_2O_2$. Roots were stained with trypan blue in lactophenol (phenol, lactic acid, glycerol, distilled water in a 1:1:2:1 ratio) by immersion in the staining solution and gently heating for 5–10 min on a hot plate. The roots were destained in clear lactophenol overnight then stored in lactophenol in small screw-top jars until analyzed.

Stained roots were placed on glass slides under cover slips and examined at 100× under brightfield optics. Mycorrhizal formation on all fine roots within each of 10 microscope fields was visually estimated and categorized as follows: (0) 0%, (1) <10%, (2) 10–33%, (3) 34–66%, (4) 67–90%, (5) >90% of field infected. In other words, if 20% of the root length in the microscope field were infected, then that field was categorized as a 2 (10–33% infection). Categories 4 and 5 were combined for analysis and are referred to as heavy infection. Category 1 (<10% of fields with infection) is referred to as light infection. The data for each sample consisted of the number of fields assigned to each category for VAM and ECM. These counts were converted to percentages. For instance, a stained sample may have seven fields with no infection, two fields in category 1 (light infection, <10% of root length in the field infected), and one in category 2 (between 10% and 33% of root length in the field infected). This sample would have 70% in category 0, 10% in category 1, 20% in category 2, and 0% in the other categories. These same categories were used for both VAM and ECM and resulted in 20 observations (two types of mycorrhizae × 10 microscope fields) for each sample. ECM infection was indicated by the presence of blue-stained sheath and/or Hartig net. VAM infection was indicated by the presence of arbuscules, vesicles, or hyphae. A magnification of 400× was used if needed to determine details of part of a field.

### *Statistical analysis*

All quantitative parameters measured during the final field readings were analyzed using factorial analysis of variance separately on each plant material type (cutting, volunteer) with a blocking factor. Size parameters were analyzed with initial height as a covariate. The plant material types (cuttings, volunteers) were analyzed separately because volunteers of *P. balsamifera* had smaller basal diameters than cuttings of the same height. A general linear model program (GLM) was used for analyses of quantitative data, while a categorical model (CATMOD) was used for categorical data such as vigor and VAM categories (SAS 1985). Type III hypothesis testing was used because of a slight imbalance in data that resulted from plant mortality and to adjust appropriately for the covariate.

Linear contrasts were used to compare soil transfers with the control, soil transfer with additional P, and both soil transfer and additional P with soil transfer. Other hypothesis tests were conducted and reported for completeness.

## Results

The mine spoil in this study area was very coarse (>50% gravel) with an alkaline pH (8.4). Spoils were also nutrient poor, with extractable $NH_4^+$ plus $NO_3^-$ <6 mg/kg and extractable P about 1.5 mg/kg. Potassium levels were 81 mg/kg while Ca levels were 1320 mg/kg. Total Na and P averaged 0.21% and 0.044%, respectively. The extractable N and P in the soil transfers were 6 mg/kg and 9 mg/kg, respectively.

Survival at the end of year 2 averaged 95% for cuttings and 98% for volunteers (Helm and Carling 1990). Volunteers were more vigorous than cuttings. Vigor had been judged by size, greenness, and overall appearance of the plants. Plant phenology did not differ among treatments.

Mean heights for the treatments on the cuttings ranged from 35.4 cm to 44.8 cm (Table 1). The main effects of P fertilization ($P<0.11$) and soil transfer ($P<0.06$) resulted in increased height of *P. balsamifera* cuttings (Table 2). However, neither amendment alone was sufficient to increase height growth compared with responses to the base level of fertilization alone ($P>0.36$ soil transfer, $P>0.44$ additional P) (Table 2). Nevertheless, application of both amendments together increased height relative to the base level of fertilization (operational control) ($P<0.02$) and base level of fertilization plus additional P fertilizer alone ($P<0.09$). There was a non-significant trend ($P<0.13$) for increased height when both amendments were applied compared to soil transfer with the base level of fertilization. No treatment had significant effect on crown area of cuttings (Table 2). Height was not significantly affected by any treatments on volunteers although additional P alone increased crown area ($P<0.09$) (Table 2).

Similarly twig lengths and current twig biomass of cuttings increased in response to main treatments of soil transfer ($P<0.07$, $P<0.06$) and P fertilization ($P<0.03$, $P<0.07$) (Tables 1, 2). Current twig growth increased in response to soil transfer or additional P or both, but none of these treatments differed from each other (Table 2). Leaf biomass did not vary with treatments for cuttings, but decreased with additional P on the volunteers ($P<0.09$) (Tables 1, 2). Most treatments had no effect on volunteers (Tables 1, 2).

**Table 1.** Twig length (cm) and oven-dried current growth (g dry matter/plant) of *Populus balsamifera* plants in response to P fertilizer and soil transfer treatments at end of second growing season. SE, Standard error of the mean; base, base fertilization level (100 mg N/kg spoil+50 mg P/kg spoil); +P, base fertilization+additional P (100 mg N/kg spoil+100 mg P/kg spoil). *n*, Number of plants measured or harvested

| Fertilization level | Soil transfer | *n* | Plant height (cm) | | Crown area ($cm^2$) | | Twig length (cm) | | *n* | Current twig mass (g) | | Leaf mass (g) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SE | Mean | SE | Mean | SE | | Mean | SE | Mean | SE |
| *Cuttings* | | | | | | | | | | | | | |
| Base | – | 28 | 35.4 | 1.4 | 271.4 | 17.8 | 13.5 | 1.1 | 13 | 0.73 | 0.1 | 3.54 | 0.2 |
| Base | + | 28 | 39.9 | 2.8 | 298.4 | 26.1 | 17.1 | 1.5 | 13 | 1.26 | 0.2 | 3.55 | 0.3 |
| +P | – | 30 | 39.1 | 2.2 | 320.6 | 30.5 | 17.7 | 1.5 | 13 | 1.24 | 0.1 | 3.13 | 0.3 |
| +P | + | 28 | 44.8 | 3.1 | 321.8 | 26.4 | 19.7 | 1.6 | 13 | 1.44 | 0.2 | 3.73 | 0.2 |
| *Volunteers* | | | | | | | | | | | | | |
| Base | – | 14 | 59.5 | 6.0 | 324.9 | 44.7 | 24.2 | 2.3 | 3 | 1.94 | 1.3 | 6.21 | 1.8 |
| Base | + | 15 | 51.6 | 6.7 | 255.2 | 33.1 | 19.4 | 2.6 | 3 | 1.44 | 0.6 | 5.82 | 2.6 |
| +P | – | 15 | 58.9 | 6.3 | 255.2 | 22.5 | 21.0 | 2.9 | 4 | 1.41 | 0.5 | 4.50 | 0.5 |
| +P | + | 15 | 52.2 | 5.4 | 273.6 | 38.7 | 21.6 | 3.3 | 3 | 2.17 | 0.7 | 4.02 | 1.0 |

**Table 2.** Significance levels of hypothesis tests for linear contrasts of above-ground physical measurements at end of year 2. * Indicates significance at 10% level or better

| Plant material | Linear contrast[a] $H_0$: a=b | Plant height | Crown area | Twig length | Twig mass | Leaf mass |
|---|---|---|---|---|---|---|
| *Cuttings* | | | | | | |
| Main effects | | | | | | |
| | Soil transfer=no soil transfer | 0.06* | 0.69 | 0.07* | 0.06* | 0.28 |
| | +P=no+P | 0.11 | 0.17 | 0.03* | 0.07* | 0.82 |
| Comparisons with control (base level) | | | | | | |
| | Soil transfer alone=control[b] | 0.36 | 0.64 | 0.12 | 0.06* | 0.97 |
| | +P alone=control | 0.44 | 0.25 | 0.06* | 0.07* | 0.37 |
| | Both=control | 0.02* | 0.22 | 0.01* | 0.01* | 0.54 |
| Individual treatments | | | | | | |
| | Soil transfer alone= +P alone[b] | 0.86 | 0.49 | 0.76 | 0.96 | 0.35 |
| Combined versus single treatments | | | | | | |
| | Both=soil transfer alone[b] | 0.13 | 0.45 | 0.22 | 0.45 | 0.56 |
| | Both= +P alone | 0.09* | 0.93 | 0.34 | 0.42 | 0.13 |
| *Volunteers* | | | | | | |
| Main effects | | | | | | |
| | Soil transfer=no soil transfer | 0.72 | 0.76 | 0.65 | 0.68 | 0.31 |
| | +P=no+P | 0.89 | 0.44 | 0.78 | 0.98 | 0.09* |
| Comparisons with control (base) | | | | | | |
| | Soil transfer alone=control[b] | 0.15 | 0.16 | 0.17 | 0.58 | 0.48 |
| | +P alone=control | 0.20 | 0.09* | 0.22 | 0.43 | 0.13 |
| | Both=control | 0.73 | 0.45 | 0.61 | 0.79 | 0.10* |
| Individual treatments | | | | | | |
| | Soil transfer alone= +P alone[b] | 0.88 | 0.74 | 0.90 | 0.75 | 0.17 |
| Combined versus single treatments | | | | | | |
| | Both=soil transfer alone[b] | 0.27 | 0.52 | 0.38 | 0.46 | 0.13 |
| | Both= +P alone | 0.35 | 0.34 | 0.46 | 0.35 | 0.36 |

[a] Treatments being compared are found on either side of the equal sign
[b] Indicates primary linear contrasts being tested. Other linear contrasts are included for completeness

Diameters and other measurements did not differ for either the base level plus soil transfer or base level plus additional P treatment. Basal diameters of cuttings averaged 9.4 mm while those of volunteers averaged 7.4 mm. Rooting depth of cuttings averaged 16.6 cm while that of volunteers averaged 22.3 cm. In some cases the entire root system could not be excavated because portions had grown between rocks.

Soil transfer treatments produced leaf N concentrations in cuttings that were 20% greater than base level treatments ($P<0.01$) (Tables 3, 4). Nitrogen concentrations were greater in cuttings growing in soil-transfer treated plots than in plots treated with additional P ($P<0.04$, Table 4). Foliar Ca concentrations were greater in cuttings when soil transfer was applied compared to the control ($P<0.09$) (Table 4). These Ca

**Table 3.** Concentrations of nutrients (%) in leaf tissues of *P. balsamifera* in response to fertilizer and soil transfer treatments at end of year 2. $n = 3$ composite samples

| Ferti-lization level | Soil transfer | N | | P | | K | | Ca | | Mg | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| *Cuttings* | | | | | | | | | | | |
| Base | – | 1.00 | 0.08 | 0.13 | 0.01 | 0.73 | 0.10 | 1.04 | 0.04 | 1.17 | 0.12 |
| Base | + | 1.26 | 0.06 | 0.13 | 0.01 | 0.71 | 0.02 | 1.14 | 0.01 | 1.21 | 0.05 |
| +P | – | 1.09 | 0.02 | 0.14 | 0.01 | 0.82 | 0.03 | 1.12 | 0.01 | 1.09 | 0.02 |
| +P | + | 1.21 | 0.02 | 0.15 | 0.01 | 0.73 | 0.04 | 1.09 | 0.03 | 1.04 | 0.07 |
| *Volunteers* | | | | | | | | | | | |
| Base | – | 1.45 | 0.09 | 0.15 | 0.02 | 0.82 | 0.11 | 1.53 | 0.03 | 1.10 | 0.06 |
| Base | + | 1.56 | 0.05 | 0.14 | 0.03 | 0.75 | 0.12 | 1.35 | 0.09 | 1.16 | 0.05 |
| +P | – | 1.21 | 0.11 | 0.17 | 0.05 | 0.76 | 0.08 | 1.20 | 0.12 | 0.95 | 0.08 |
| +P | + | 1.34 | 0.15 | 0.14 | 0.04 | 0.81 | 0.10 | 1.17 | 0.16 | 0.93 | 0.18 |

**Table 4.** Significance levels of hypothesis tests for linear contrasts of foliar nutrient concentrations at end of year 2. * Indicates significance at 10% level or better

| Plant material | Linear contrast[a] $H_0$: a = b | N | P | K | Ca | Mg |
|---|---|---|---|---|---|---|
| *Cuttings* | | | | | | |
| Main effects | | | | | | |
| | Soil transfer = no soil transfer | 0.01* | 0.66 | 0.26 | 0.44 | 0.96 |
| | +P = no+P | 0.71 | 0.39 | 0.26 | 0.71 | 0.17 |
| Comparisons with control | | | | | | |
| | Soil transfer alone = control[b] | 0.01* | 0.75 | 0.70 | 0.09* | 0.75 |
| | +P alone = control | 0.26 | 0.54 | 0.23 | 0.14 | 0.50 |
| | Both = control | 0.02* | 0.36 | 1.00 | 0.43 | 0.30 |
| Individual treatments | | | | | | |
| | Soil transfer alone = +P alone[b] | 0.04* | 0.75 | 0.13 | 0.77 | 0.33 |
| Combined versus single treatments | | | | | | |
| | Both = soil transfer alone[b] | 0.51 | 0.54 | 0.70 | 0.29 | 0.19 |
| | Both = +P alone | 0.12 | 0.75 | 0.23 | 0.43 | 0.69 |
| *Volunteers* | | | | | | |
| Main effects | | | | | | |
| | Soil transfer = no soil transfer | 0.24 | 0.77 | 0.91 | 0.48 | 0.89 |
| | +P = No+P | 0.06* | 0.98 | 0.86 | 0.05* | 0.16 |
| Comparisons with control | | | | | | |
| | Soil transfer alone = control[b] | 0.51 | 0.77 | 0.66 | 0.30 | 0.74 |
| | +P alone = control | 0.11 | 0.91 | 0.52 | 0.06* | 0.41 |
| | Both = control | 0.51 | 0.82 | 0.46 | 0.07* | 0.34 |
| Individual treatments | | | | | | |
| | Soil transfer alone = +P alone[b] | 0.04* | 0.85 | 0.84 | 0.28 | 0.26 |
| Combined versus single treatments | | | | | | |
| | Both = soil transfer alone[b] | 0.21 | 0.94 | 0.69 | 0.30 | 0.22 |
| | Both = +P alone | 0.29 | 0.91 | 0.55 | 0.95 | 0.89 |

[a] Treatments being compared are found on either side of equal sign
[b] Indicates primary linear contrasts being tested. Other linear contrasts are included for completeness

concentrations did not differ when individuals receiving soil transfer alone or additional P alone were compared ($P > 0.77$) (Table 4). P, K, and Mg levels were not significantly different among treatments (Tables 3, 4). Addition of P to volunteers tended to reduce leaf N and Ca concentrations (Tables 3, 4).

Soil transfer treatments on cuttings resulted in more ECM formation than the controls ($P < 0.01$) or P alone ($P < 0.08$). Greater ECM formation resulted on cuttings when both soil transfer and additional P were applied compared with ECM formation when only additional P was applied ($P < 0.03$) (Fig. 1). However, additional P did not change the percentage of ECM tips compared to the control ($P > 0.34$), nor did additional P combined with soil transfer alter the percentage of ECM tips compared with soil transfer alone ($P > 0.63$). Soil transfers increased the relative percentage of brown wooly ECM ($P < 0.08$) but reduced the tan types ($P < 0.08$) compared to the control. Most other comparisons involving ECM formation were not significant. Volunteers had more types of ECM when no treatment was applied (Fig. 1). Reasons why treat-

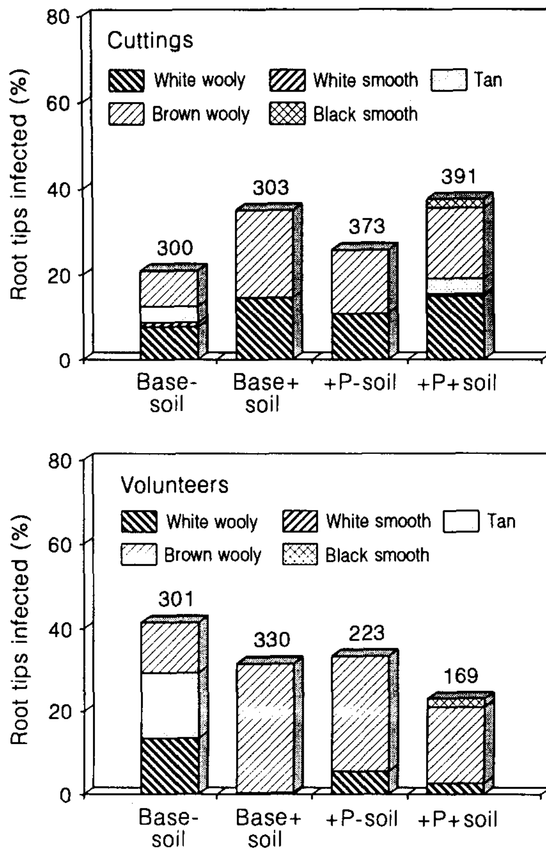


**Fig. 1.** Percentage of root tips infected by ectomycorrhizae on cuttings and volunteers of *Populus balsamifera* at the end of year 2. Treatments: *Soil*, soil transfer; *Base*, 100 mg/kg N+50 mg/kg P; +*P*, 100 mg/kg N+100 mg/kg P. *Numbers* above each bar are mean number of root tips examined per plant. SE of mean for percent infected root tips: 3% for cuttings and 10% for volunteers. Estimates were made on unstained samples under a dissecting microscope at magnification ×40. The different types may represent different fungal species or age classes or both

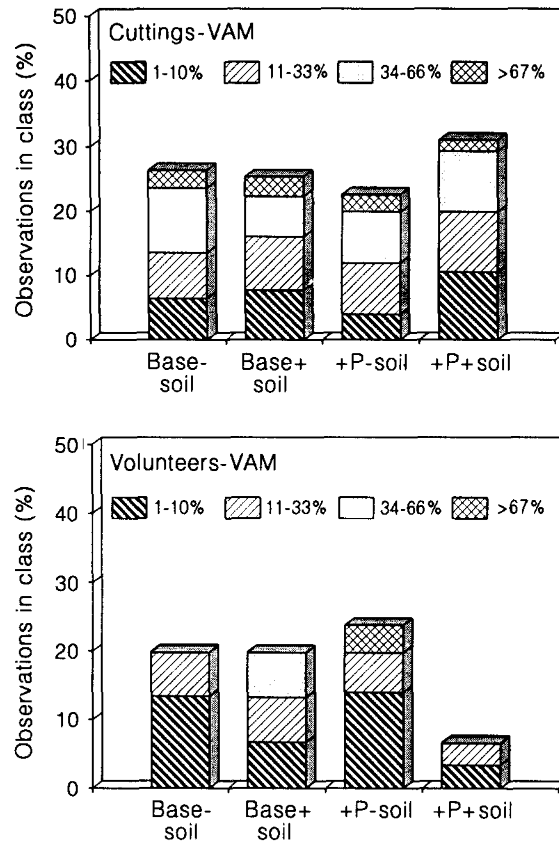


**Fig. 2.** Percent of observations in each infection percentage class for VAM in harvested plants at end of year 2. Estimates were made on stained samples using a compound microscope at magnification ×100

ments appeared to reduce the diversity of types on volunteers are not known, but only three root systems of volunteers were examined for each treatment. These morphological categorizations should not be confused with fungal species identifications. Different mycorrhizal fungal species may form mycorrhizae that appear the same. Different ages of the same mycorrhizal fungi on the same roots may have different appearances.

Mycorrhizal formation on plants harvested because they were more vigorous or larger than other plants was not noticeably different from the means for their category. Some had greater infection, some had less infection, and some were about the same.

Both VAM and ECM were observed on the cleared and stained samples. Only the VAM results are reported for the stained roots, since the root tip count was considered a better representation of ectomycorrhizal infection. Approximately 26% of the microscope fields examined for cuttings contained at least some VAM infection while 18% of the fields examined for volunteers contained some VAM infection (Fig. 2). VAM infection levels on cuttings were similar among treatments (Fig. 2). None of the variation in VAM on cuttings or on volunteers was significant.

Densities of VA spores were low, averaging about 1.2 spores/g spoil in the rooting zone of the cuttings and 2 spores/g spoil near volunteer roots. Spore densities for individual plants ranged from 0.1 to 14.2 spores/g spoil (Helm and Carling 1990).

## Discussion

Although soil transfer improved current twig growth, additional P was needed besides the soil transfer to increase plant heights of *P. balsamifera* cuttings after 2 years in the field. There were no significant differences between physical parameters of *P. balsamifera* cuttings when treatments with soil transfer and additional P were compared to each other. Hence, soil transfer could be substituted for additional P with no negative growth impacts. However, soil transfer had significant positive effects on leaf N concentrations and ECM formation where additional P did not. The combination of additional P plus soil transfer treatments did not suppress ECM formation below levels established with soil transfer alone.

Increased mycorrhizal formation resulting from the soil transfer may have long-term benefits that cannot be achieved with fertilizer treatments. Fertilizers normally would be depleted in a few years. Once established, mycorrhizae presumably would be active each year and would help re-establish ecosystem functions.

The increased levels of mycorrhizal formation in response to soil transfer support the hypothesis of mycorrhizal effects. However, some of the increased growth in response to soil transfer may have resulted from other soil microorganisms or soil properties. Effects of bacteria may increase or decrease mycorrhizal formation (Bowen and Theodorou 1979). Bacteria present in the soil also may solubilize phosphates (Azcon et al. 1976), enhance P uptake, or may fix $N_2$ in the rhizosphere (Li and Hung 1987; Amaranthus et al. 1990).

Mycorrhizae may be particularly important for establishment of plant species such as *P. balsamifera,* which grow rapidly when nutrients are not limiting but slowly on nutrient poor sites. Chapin et al. (1983, 1986) reported substantial increase in height of *P. balsamifera* with increased P in the laboratory. Heilman (1990) found that *P. trichocarpa* did not increase growth or foliar P content in response to P fertilization on Mount Saint Helens. It grew best on high-N soils but could not compete with *Alnus rubra* on low-N soils. Mycorrhizae appeared to be at least partially responsible for the increased growth of outplanted cuttings in our study. The increased nutrient absorption associated with mycorrhizal formation would be expected to increase plant growth, especially on young, developing plants such as these.

Soil transfers did not appear to aid the volunteers evaluated within the period of this study although growth tended to increase with soil transfer alone compared with the control ($P<0.15$ height, $P<0.16$ crown area, $P<0.17$ twig length). Several plausible explanations exist for this lack of response. (1) *P. balsamifera* volunteers may sprout from rhizomes connected to other *P. balsamifera* individuals and have fewer feeder roots compared with the fibrous roots of cuttings. These connections to parent plants may aid volunteer *Populus* individuals in obtaining nutrients, therefore reducing the need for mycorrhizal fungi. (2) Physical disturbance of the rooting zone prevented good contact between spoil and root. The controls also had base levels of fertilizer placed in the rooting zone so their roots were disturbed. However, the root fragments in the soil being transferred were fairly stiff, a characteristic that may have created minor gaps between the spoil and roots. (3) The existing rhizosphere community was biologically disrupted by the introduction of different fungal species, and the environmental conditions were changed by the addition of P. (4) Allelopathic effects may be present. Leachates from *P. balsamifera* leaves have been shown to suppress growth and nodulation of *Alnus* (Jobidon and Thibault 1982). *P. balsamifera* also has chemical defenses against herbivory (Bryant and Kuropat 1980). Perhaps *P. balsamifera* root exudates present in volunteers, but not young cuttings, affect mycorrhizal colonization. These effects may not be present in rooted cuttings, which would be physiologically younger individuals. Their roots would be less lignified compared to those of 5- to 10-year-old volunteers.

Of these alternative explanations, we speculate that the first hypothesis is the most plausible. A rhizomatous network would reduce the plant's need for mycorrhizae and nutrient transfers that occur among the hyphae. Nutrients and carbon may be transferred along mycorrhizal hyphae connecting plants, even when they are different plant species (Harley and Smith 1983; Finlay and Read 1986a, b). This may be supported by the fact that control volunteers had greater foliar N concentration than any treatments on cuttings. Corresponding treatments on volunteers had greater N than cuttings. Although these coarse rhizomes and adventitious roots can reach greater distances from the parent plant, they have small surface areas for absorbing nutrients. Stabilization of the spoil adjacent to the plant would be less compared to that around vigorous, fibrous roots of cuttings. However, the network of underground connections is important for spoil stabilization on a larger scale.

Responses of plant species and mycorrhizae to fertilizer depend on existing environmental conditions, especially the soil nutrient levels. Studies have shown mixed results with respect to suppression of mycorrhizal formation with additional P fertilization. Some show no effect while others show that high levels of P suppress mycorrhizae (Parkinson 1984; Carling et al. 1989). Some indicate that mycorrhizae and bacteria are both needed for the use of some forms of P, such as rock phosphate (Azcon et al. 1976). Parkinson (1984) demonstrated that additional levels of a full fertilizer (N:P:K) could suppress mycorrhizal infection of *Pinus banksiana* (jack pine) seedlings growing in peat. Some mycorrhizal fungal species infected the roots only when no fertilizer was applied. Similarly, low fertilizer levels permitted high levels of VAM infection in *Agropyron trachycaulum* (slender wheatgrass), but additional levels completely suppressed VAM formation (Parkinson 1984). Allen and Allen (1990) hypothesized that plant species in early successional sites would be less dependent on mycorrhizae on sites with greater levels of nutrients. Our study demonstrated similar ECM infection percentages for additional P and soil transfer but lack of suppression of ECM when additional P was applied.

Increased N levels in plants with mycorrhizae have occurred in previous studies. Ectomycorrhizal fungi produce enzymes that may increase N uptake (Melin 1953). Also, these fungi can use ammonium and some amino acids (Harley and Smith 1983) to improve the N nutrition of the plant. Free-living N-fixing bacteria also may occupy the rhizosphere of some ectomycorrhizal plants (Li and Hung 1987) and their activity has been enhanced by soil transfers (Amaranthus et al. 1990). Fogel (1980) has estimated that 43% of the cycling of N in a *Pseudotsuga menziesii* forest results from mycorrhizal fungi. Hence, increased N levels in leaf tis-

sues might be expected with soil transfers, although it may result from increased nutrient absorption by mycorrhizae, effects of bacteria, or interactions.

In summary, plant growth of *Populus balsamifera* cuttings responded similarly to soil transfer alone and to additional P alone, but both treatments were required for increased height compared to the control. However, mycorrhizal formation and leaf N concentrations were increased by soil transfers but not by additional P alone. Hence, the soil transfer technique appears to be a suitable, and in some ways a superior, substitute for high levels of P fertilization for reforestation of abandoned mined lands, especially in regions such as Alaska where transportation, labor, and materials such as fertilizer may be expensive. Future research is required to improve understanding of responses to different soil-transfer sources, plant species, and fertility levels and other factors affecting mycorrhizal formation.

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