PHOSPHATE FERTILISER AND COPPER NUTRITION OF MARITIME PINE IN SOUTH-WESTERN FRANCE

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ABSTRACT

Several studies have been published, mostly in French, concerning the effects of phosphorus nutrition on copper movements through the soil/micro-organism/plant system by endogenous and extraneous regulation. All experiments involved maritime pine (*Pinus pinaster* Soland in Ait.) growing on the typical sandy soil (hydromorphic humic podzol) representative of nearly 60% of the pine plantation in Landes de Gascogne. Phosphate fertiliser affects copper solubility in the soil chemically, by soil micro-organism changes, or by root exudate modification, and it also affects copper uptake and translocation from root to shoot, including xylem sap chemistry in field-grown trees. The level of copper nutrition is important and understanding of the physiological interaction has practical consequences for pine fertiliser treatment and copper deficiency monitoring.

Keywords: phosphorus; copper; fertiliser; mineral interaction; foliar analysis; xylem sap; *Pinus pinaster*.

INTRODUCTION

The forest of Landes de Gascogne is the largest plantation forest in Europe. The total *Pinus pinaster* plantation area established to date is 1.03 million ha and annual commercial logging was 6.7 million m^3 in 1988. From 1950, more intensive production using modern silviculture techniques commenced with draining, soil preparation including ploughing, fertiliser, and vegetation control in the early and late stages of the plantation. During the same period, several growth disturbances and malformations without pathological explanation appeared in pines, and particularly in 3-year-old trees—twisting of branches and leaders, large numbers of fascicle shoots, loss of apical dominance, die-back of the top, and production of unlignified tissues. The symptoms were similar to those described for other coniferous species with copper deficiency (Downes & Turvey 1992; Saur 1990c) and occurred irregularly in spring time depending on the weather conditions in the current year. The similarity of the symptoms to those in other pine species, and the very low level of trace elements in these sandy acid soils (Saur & Juste 1993) and in an adult stand (Saur *et al.* 1992), were consistent with a possible problem of copper nutrition induced or aggravated by phosphorus fertiliser.

The aim of this paper is to present an overview of several studies describing effects of phosphorus on copper movement through the soil solution and the plant by endogenous and extraneous regulation.

MATERIALS AND METHODS

Most of the results presented have been published in French with full details on experimental operations and statistical analysis. Appropriate references for each phase of the study are given later.

The experiments included field trials, pot experiments, and soil incubation, and all occurred on typical sandy soil from hydromorphic humic podsol, representative of nearly 60% of the pine plantation in Landes. The soil was an acid sandy soil (97% coarse sand) known to be extremely deficient in phosphorus (29 mg P/kg solids in 2% citric acid extractable form) and copper (2.3 mg Cu/kg solids in total form, Aqua Regia) and rich in organic matter (8.5% dry weight (DW)). The upper 0–20 cm, excluding litter, had a pH of 3.9 (for a more detailed chemical and physical description of this soil, *see* Saur 1989c).

All the water-culture experiments used the same nutrient solution but with different phosphorus or copper levels. The standard nutrient solution was continuously aerated and contained the following (mM): 2 NH_4NO_3, 0.5 KH_2PO_4, 0.25 $CaCl_2$, 0.25 $MgSO_4$, 0.1 Fe, 8.0 10^{-3} B, 1.5 10^{-3} Mn, 0.15 10^{-3} Zn, 0.0015 10^{-3} Co, 0.0015 10^{-3} Mo (pH=4.5).

All cultures were obtained from *Pinus pinaster* seeds of the "Landaise" geographic race (south-west of France).

Analysis of variance was used to determine significant effects of treatments on measured parameters and means were compared by appropriate means comparison tests. Most of the statistical calculations were done with SAS program and INRA programs and have been presented previously.

PRIMARY OBSERVATION

An early study developed to demonstrate the effect of phosphate fertiliser on the main micro-nutrients (manganese, zinc, copper, and boron) in commercial plantations indicated the largest response in the foliar concentration of copper (Saur 1989b).

Method: the study concerned three different-aged plantations.

- Seedlings (6 months old): four treatments (0 (control), 33, 65, and 164 kg P/ha) were replicated five times. Each replicate consisted of 3.5 kg soil potted with three seedlings.
- Juvenile pines (3 years old): three treatments (0 (control), 13, and 26 kg P/ha) were replicated three times in a randomised block trial. Needles were collected from 10 trees for each treatment.
- Adult pine: two treatments (0 (control) and 50 kg P/ha) were replicated three times. Needles were collected from 10 trees for each treatment.

All the needles were sampled on the 1-year-old main shoot of the 3-year-old whorl of the trees in February when the concentrations are known to be in steady state.

Results: Copper concentrations decreased substantially with the increasing age of control trees (Fig. 1). Phosphate fertiliser produced a statistically significant effect on copper by decreasing needle concentrations, even in adult plantations where fertiliser was last applied more than 10 years prior to sampling. In the 6-month-old seedlings the total copper uptake was estimated and varied from 17 μg/plant in the controls to 7 μg/plant in the 164 kg P/ha treatment, excluding a dilution effect.

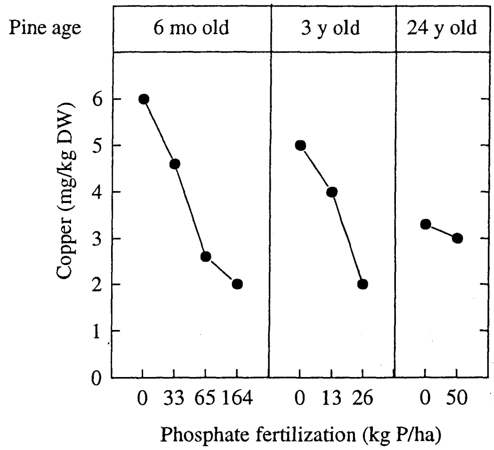

FIG. 1–Copper concentration in needles in relation to soil fertiliser treatment for three different *Pinus pinaster* experiments on the same type of soil.

The effect of phosphate fertiliser on copper concentration was thus established. Foliar analysis, however, in plantations where growth disturbance occurred, did not demonstrate an obvious relationship between copper levels in needles and growth rate or stem deformations (unpubl. data).

EXPERIMENTAL APPROACH

The goal of the following studies was to examine the level of antagonism between phosphate fertiliser and copper movement through the soil-plant system in order to understand the physiological regulation of copper deficiency or sub-deficiency occurring during only a short period in spring time.

We hypothesise that the antagonism could occur through (i) availability of copper by soil solution equilibrium, microbial activity, or root exudate modification, (ii) copper uptake by the root system, (iii) copper translocation from roots to above-ground parts.

Copper Bioavailability in the Soil

Effect of phosphate on soil and micro-organisms

In order to determine the direct effect of phosphate on the copper level of the soil solution available to the plant, soils were incubated for short periods of time (4 hours agitating—Saur 1990d) or longer periods of time (6 months—Saur 1990b) with naturally occurring micro-organisms and sterile conditions.

Methods:

- Short incubation: 50-g samples of dry soil were thoroughly mixed with 100 ml deionised water and 2 mM P of phosphoric acid, calcium-, sodium-, and potassium-phosphate solutions for 4 hours. Water-soluble copper was determined by electrothermic atomic absorption spectrophotometry with a HNO_3-Palladium modifier perfected in our laboratory (Saur & Gomez 1989). The phosphorus content and pH were monitored in the same solutions.
- Long incubation: 4.5-kg soil pots were maintained for 6 months in greenhouse conditions with daily automated watering to 60% of water retention capacity. Four levels of superphosphate (5, 10, 15, 20 mg P/kg) were initially applied on both fresh and chloroform-sterilised soils. Copper extraction and determination were carried out as for short-term incubation.

Results: Phosphate had no significant effect on water-soluble copper level after short-term or sterile long-term incubation (Fig. 2). On the other hand, in soil incubated with the naturally occurring micro-organisms phosphate increased copper levels markedly. Soil pH was not affected by phosphate application and copper levels measured in fertiliser for a possible source of contamination were negligible. This study indicated that phosphate fertiliser increases the copper level in well-watered soil solutions, not by chemical effects but by changing the microbial activity.

Some similar copper determinations on the upper soil layers of phosphorus fertiliser trials indicate that this effect also occurs under field conditions (unpubl. data).

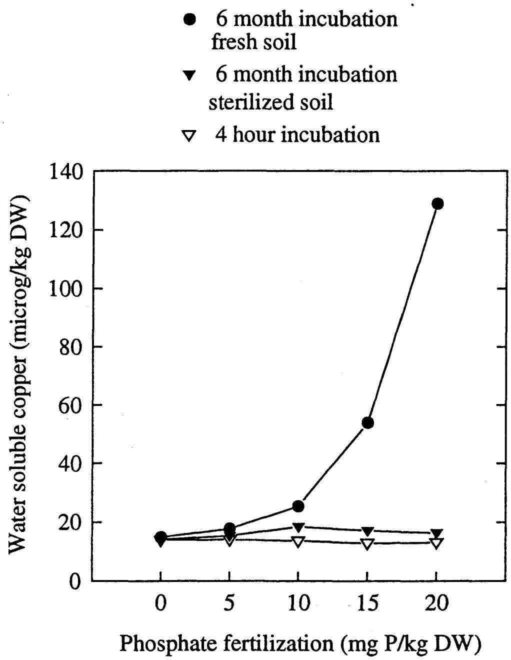

FIG. 2–Water-soluble copper concentration in soil in relation to phosphate fertiliser treatment, after 4 hours' incubation and after 6 months' incubation in sterilised and unsterilised soil.

Effect of phosphate nutrition on root exudation

It has been suggested for many years that root exudates may affect metal speciation in the soil solution. Root exudates are influential in increasing soil cation solubility by changing pH and redox conditions, and it is commonly assumed that chelation by root exudates makes a significant contribution to trace element solubilisation and acquisition. The direct effect of *P. pinaster* on copper solubility was demonstrated by Saur & Gomez (1989) who used root exudate solution from pine hydroponics culture as an extracting reagent on soil. Soluble-copper concentrations were markedly increased by exudate solution compared to deionised water and testified to the effectiveness of root exudates on copper mobilisation independent of microbial activity.

Consequently, the effect of phosphate fertiliser on the ability of root exudate to mobilise copper could be considered (Saur 1990d).

Methods: Young seedlings were cultivated for 1 month in a hydroponic system with four levels of phosphorus (0, 1, 2, 3 mM). Root exudates were collected in deionised water for 24 hours and adjusted to 12 g C/ℓ, 50-g samples of dry soil were thoroughly mixed with 100 ml deionised water and exudate solutions for 4 hours, and soluble copper was determined.

Results: The level of phosphorus supply of the plant drastically affected the ability of the root exudates to solubilise copper from the soil (Fig. 3). This antagonism between phosphorus nutrition and copper solubility is the result of qualitative changes in exudate composition because the total amount of exudate carbon and the pH of the solutions was not affected.

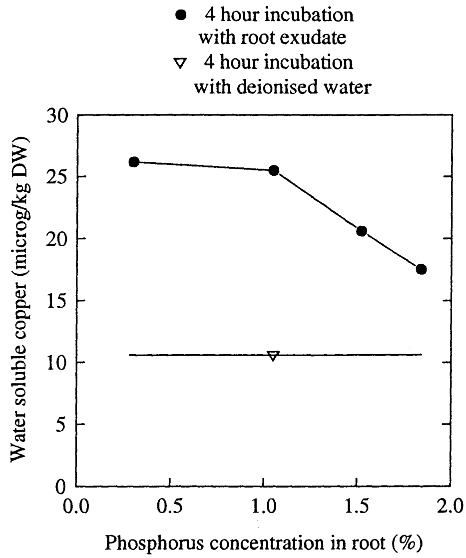

FIG. 3–Soluble copper concentration in soil extracted by deionised water or root exudate solutions (12 mg C/ℓ) in relation to the phosphorus concentration of the roots used for the exudate collection.

Effect of phosphate on root-soil system

Pot experiments were conducted to compare the previous results with a less artificial model which included the root system and associated micro-organisms, e.g., mycorrhizas, with a permanent contact with the soil (Saur 1989a).

Methods: 4.5-kg soil pots were maintained for 6 months in greenhouse conditions with daily automated watering to 60% of water-retention capacity. Two rates of superphosphate (10 and 20 mg P/kg) were applied to control pots (containing no plants) or to pots containing one tree per pot. Copper extractions and determinations were conducted at the end of the culture. The degree of ectomycorrhizal formation was estimated as the percentage of mycorrhizal short roots to total short roots (Saur 1993).

Results: The increase in soluble copper caused by phosphate fertiliser as previously demonstrated was confirmed (Fig. 4). The significant difference between copper in the controls and in the soil with plants demonstrated the effect of the mycorrhiza-pine symbiosis on the water-soluble copper. The degree of mycorrhizal formation was not affected by phosphorus fertiliser and was due mainly to the presence of *Telephora terrestris*. Phosphate fertiliser reduced the relative increase of water-soluble copper in the soil in the presence of a plant from 150% with 10 mg P/kg to 32% with 20 mg P/kg.

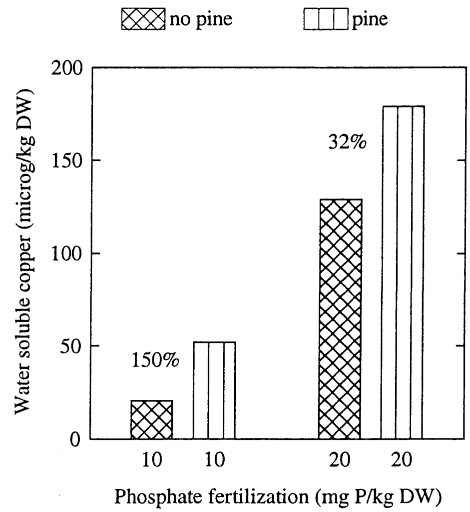

FIG. 4—Water-soluble copper concentration in soil after 6 months' incubation with or without pine culture, in relation to phosphate fertiliser (the percentage indicates the relative gain in copper in the presence of pine).

In conclusion, in spite of the decrease in copper solubility with the root exudates from plants high in phosphorus, the overall effect of phosphorus fertiliser is to increase soluble copper. The soil level interaction cannot obviously explain the antagonism between phosphate fertiliser and copper content in needles and the following experiments were aimed to localise negative interaction in the copper flux through the plant.

Plant Copper Uptake

We hypothesised that phosphate nutrition could influence copper uptake through a direct effect on the soil solution or through physiological effects of phosphorus metabolism on the copper absorption process. Two water-culture experiments were designed to distinguish between these effects.

Methods: Copper uptake of 1-month-old seedlings was measured after 24 hours in 8 μM Cu solution (Saur 1990d). This was measured with different phosphorus concentrations in the solution (0, 2, 3 mM P, as potassium- and sodium-phosphate) and with seedlings with various root phosphorus concentrations obtained by culturing for 15 days in different solutions (0, 1, 2, 3 mM P).

Results: Phosphate in solution did not disturb copper uptake (not significantly different from 19 ng Cu/plant); this confirmed the assessment of Graham (1981) who reported no dramatic effect of phosphate ion on copper uptake by the plant. On the other hand, increased phosphorus concentration in root tissues strongly inhibited copper uptake (Fig. 5).

These results provide evidence for metabolic control of copper uptake via phosphate nutrition and were consistent with the final antagonism observed in the long-term experiment.

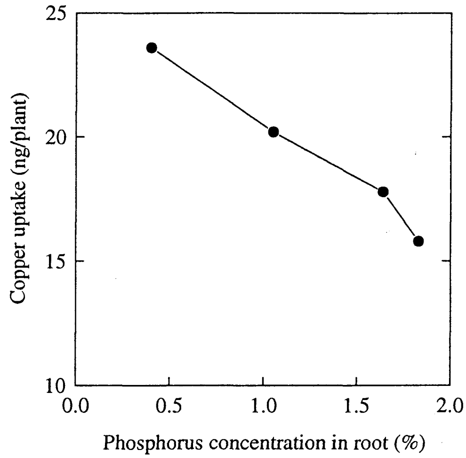

FIG. 5–Copper uptake by pine during 24 hours in 8 μM Cu solution, in relation to the phosphorus concentration in the roots.

Copper Translocation from Root to Shoot

The last level of regulation analysed was the translocation of copper from root to shoot, including the loading of xylem sap by a metabolically active process through the endodermis. Three experiments were conducted involving very young seedlings in water culture, small trees in pot culture, and adult trees in the field.

Water culture

Translocation was estimated after loading the root system with a high level of copper and submitting the plants to different levels of phosphate nutrition (Saur 1990a–d, and unpubl. data).

Methods: Roots of 15-day-old seedlings were enriched with copper by placing them in a 0.16-mM Cu solution for 24 hours. The seedlings were subsequently cultivated for 15 days in solutions containing 1, 2, or 3 mM P before they were harvested and their copper content was determined.

Results: The results are expressed in terms of the amount of translocated copper *v.* the phosphorus concentration in the root tissue as a consequence of increasing levels of phosphate in the culture solution (Fig. 6). The antagonism between phosphorus nutrition and copper translocation was clearly demonstrated and the growth rates of the plants in the different phosphorus treatments during the 2 weeks of the experiment were not significantly different.

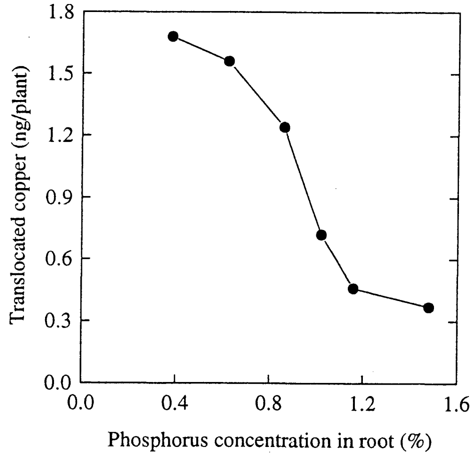

FIG. 6–Copper translocated from root to shoot by pine during 15 days after 24 hours' copper loading in 160 μM Cu solution, in relation to the phosphorus concentration in the roots.

Pot culture

Methods: Pines were planted in 4.5-kg soil pots and maintained for 6 months in a greenhouse with daily automated watering to 60% of water-retention capacity. Three rates of superphosphate (10, 20, 50 mg P/kg) were applied (Saur 1989c, 1990a).

Results: Copper content in the root system increased and in the above-ground parts decreased with increasing phosphorus concentration in the root (Fig. 7). This was consistent with the

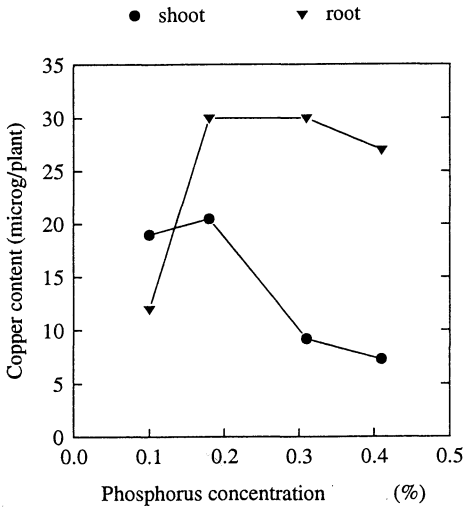

FIG. 7–Copper content in root and shoot tissue of 6-month-old pine in relation to phosphorus concentration in the root.

results from the water culture experiment and indicated that the main level of antagonism appeared to be the translocation from root to shoot. The increase in the copper content of roots was due to an increase in soil soluble copper, despite an inhibition of the copper uptake rate.

In order to extrapolate these results to forest conditions, a direct approach to examine copper transfer in large trees was required. Collecting xylem sap was preferred to mineral content studies which are not precise enough for this purpose, and foliar copper concentrations are not correlated with deficiency symptoms. Xylem sap analysis also allows an instantaneous diagnosis and copper nutritional problems occur in a very short period of time during shoot elongation.

Xylem sap on field trees

Trunk xylem sap collection was conducted in a plantation fertiliser trial by harvesting complete trees before the first thinning operation (unpubl. data).

Methods: The field layout consisted of 32 × 32-m plots. Triple superphosphate (45%) was applied at 13 and 26 kg P/ha before ploughing and direct sowing at 10 000 seeds/ha in April 1985. The trees were culled in 1988 and 1991 to a final spacing of 4 × 2 m. Samples were randomly collected in three contiguous plots (control and phosphorus-treated) on 17 June and 20 August 1992, with six replicates for each date × treatment. Xylem sap was collected by water displacement in a 0.5-m basal trunk segment under a pressure of 10^4 Pa. The first fraction, contaminated by wood injury and previously estimated to 40 ml, was discarded. A pure sap sample (80 ml) was immediately filtered and frozen for conservation. Xylem sap was analysed for phosphorus by inductively coupled plasma spectrophotometry and for copper by electrothermic atomic absorption spectrophotometry with Zeeman effect in 2% HNO_3 solution.

Results: Phosphorus levels were significantly higher in response to fertiliser (Fig. 8) and the greater contrast occurred in June during shoot elongation. Copper concentrations were significantly decreased by phosphorus fertiliser. The difference was maximum in spring time, with a 47% reduction in xylem sap copper concentration in June. August sampling occurred after shoot elongation and during needle expansion. These results confirm the antagonism and give an indication of its importance during shoot elongation, which is the critical stage for stem growth disturbance.

CONCLUSIONS

The main levels of interaction between phosphate fertiliser and copper transfer through the soil-plant system are summarised in Fig. 9. Phosphate in the soil has a synergistic effect on copper mobilisation from the solid phase via micro-organism activity. Internal phosphate status acts in an antagonistic way on the ability of root exudate to mobilise soil copper, on the absorption process, and on the translocation from root system to above-ground growing tissues. The results of these combined levels of interaction are increased copper content in the roots and decreased copper content in above-ground parts as a result of phosphate fertiliser (Fig. 9). For *P. pinaster* growing in this edaphic condition, the copper supply in the above-ground part is not dependent on copper supply in soil; it is dependent on the phosphate level in the plants and the effect is mainly confined to root-shoot xylem transfer. Copper transfer is strongly controlled by phosphorus metabolism and the consequence is a strong

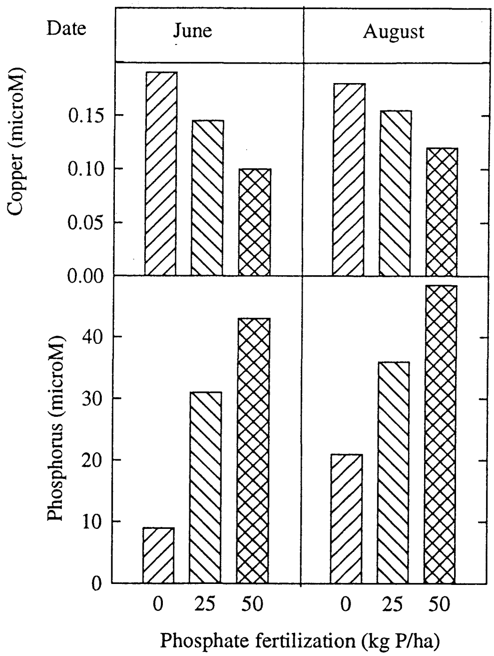

FIG. 8–Phosphorus and copper concentration in stem xylem sap in relation to phosphate fertiliser for two dates.

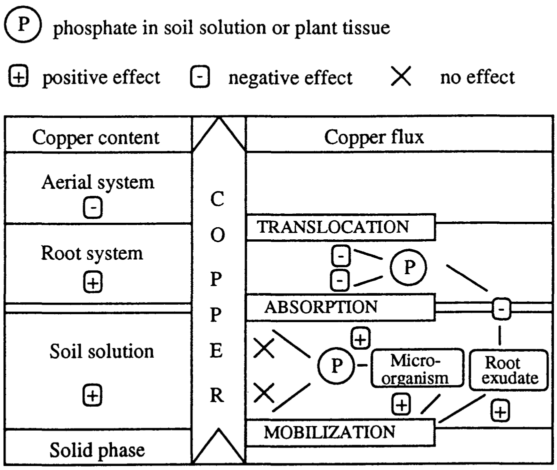

FIG. 9–Effects of phosphate fertiliser on copper nutrition.

correlation between phosphorus concentration and copper, as illustrated by 6-month-old pines (Fig. 10) grown in contrasting conditions that included phosphate and calcium carbonate fertiliser with different levels of organic matter (Saur 1989c, 1990a).

Jarvis (1981) suggested that an increasing supply of phosphate from the soil may restrict transport of copper from roots to shoots. This interaction, previously unknown in forest

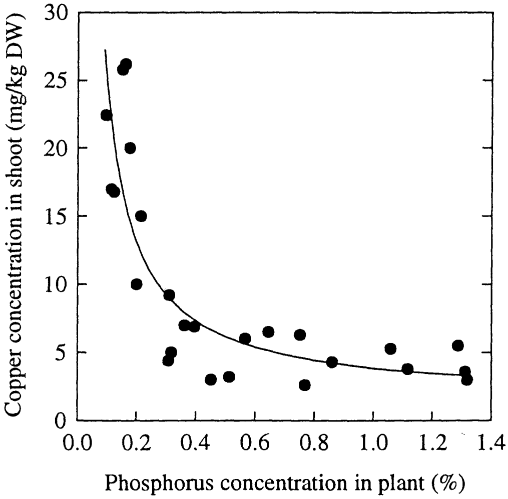

FIG. 10–Copper concentration in relation to phosphorus concentration in 6-month-old pine in various soils and fertiliser treatments. The curve is fitted by y = 2.308/x+1.62

nutrition (Ballard 1986), is a new example and tends to generalise the idea expressed by Adams (1986) who reported that phosphorus-induced copper deficiency (and phosphorus-alleviated copper toxicity) seemed to be confined to citrus. The level of interaction we isolated in the soil-plant experimental approach is consistent with the idea that copper uptake and translocation are metabolically mediated processes (Mengel & Kirkby 1987) and are markedly affected by the presence of other ions (Graham 1981).

The practical consequence is that increasing the supply of copper to the plant is not possible by application of copper fertiliser because it produces toxicity symptoms on the root before changing the nutrition of shoots (Saur 1990b). There are two ways which should be explored to correct copper deficiency—(i) foliar spraying (Saur 1991), and (ii) a copper-phosphate fertiliser balance with particular attention to reducing the phosphate rate (Saur 1993).

Xylem transport seems to be the key process in phosphate-copper antagonism and its importance is stressed by xylem sap analysis. Xylem analysis appears to be a convenient tool for monitoring copper nutrition disorder in pine plantations of south-west France. Foliar analysis is not accurate enough for diagnosis of copper deficiency in young plantations and nutritional growth disorders occur in a short period during spring in reaction to meteorological conditions and probably soil water status.

An extension of this study could be to conduct new experiments in plantations by monitoring xylem sap, growth, and meteorological conditions to analyse the main factors involved in copper-induced growth disturbance. Another exciting prospect would be to investigate the physiological processes involved in the "magic box" of copper translocation from root to shoot in order to define precise criteria for copper efficiency with regard to phosphorus antagonism. The goal is to produce juvenile tests for the *P. pinaster* breeding programme.

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