UPTAKE AND DISTRIBUTION OF TRUNK INJECTIONS IN CONIFERS

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Abstract. Seven coniferous species, four of them resinous, were injected with pressurized latex capsules containing 250 mL (7.5 oz) of water using different injector sizes ranging from 3 to 7 mm (0.12 to 0.28 in.) in diameter and from 25 to 70 mm (1 to 2.8 in.) in length. In resinous species, uptake was slow and sometimes incomplete, particularly in spring when resin secretion was high. Water uptake rate decreased when the smaller-diameter injectors were used. An injector with a diameter of 7 mm and length of 70 mm had the fastest rates of water uptake. In nonresinous species, the injector with a diameter of 4 mm (0.16 in.) worked as well as the more widely used 6 mm (0.24 in.) injector in most cases, with the added advantage of reducing wound size and decreasing healing time. Spanish fir (*Abies pinsapo*) trees were injected with a 50 mM solution of rubidium chloride. Rubidium (Rb^+) was recovered in all tree sections sampled for both healthy and unhealthy trees. Healthy trees had a greater and faster uptake rate than unhealthy ones. Results indicate that conifers were injected efficiently. Uptake rate is slower than in angiosperms, but distribution is more uniform. Due to this slowness, the low-pressure trunk injection methods composed of individual devices on each injection point are more efficient for injecting coniferous trees.

Key Words. Conifers; coniferous species; resinous species; resin secretion; trunk injection.

Tree injection has been practiced since as early as the 12th century, when Arabs described methods for introducing solid substances into holes or cuts in plants for imparting perfumes and medicinal qualities to fruits, or colors to flowers. Later, Leonardo da Vinci was, apparently, the first person who developed systematic experiments with liquid injections into trees (Roach 1939).

Numerous injection experiments have been carried out, and a compilation of works to the beginning of the 20th century was made by Roach (1939). The development of systemic fungicides renewed interest in tree injection in the 1970s (Kielbaso et al. 1979). Today, tree injection is an alternative method of chemical application with the following advantages: (1) efficient use of chemicals, (2) reduced environmental contamination, (3) applicable when traditional soil and foliar application methods are either ineffective or too difficult, and (4) acceptable in populated areas.

Several injection methods, including bark banding (Koehler and Rosenthal 1967), trunk infusion (Schreiber 1969), and pressurized trunk injections (Filer 1973; Helburg et al. 1973; Reil and Beutel 1976), were developed

in the second half of the 20th century. Low-pressure trunk-injection methods, working at pressures below 100 kPa, described by Darvas et al. (1984), McClure (1992), and Navarro et al. (1992), are currently popular because special equipment is not required and solutions are distributed efficiently. These injection methods differ in terms of ease of use and application cost. The method described by Navarro et al. (1992) is widely used in Spain with positive results for treatment of tree pathogens (Fernández-Escobar et al. 1994, 1999), tree insects (Fernández de Cordova and Gallego 1995, 1997), and nutritional disorders (Fernández-Escobar et al. 1993).

In addition to the injection system, other factors influence uptake and distribution of injected substances in tree trunks. These factors are related to the technique used (Nyland and Moller 1973; Sachs et al. 1977; Sánchez-Zamora and Fernández-Escobar 2000), the chemical injected (Reil 1979; Guest et al. 1994), the plant species (Sachs et al. 1977; Sánchez-Zamora and Fernández-Escobar 2000), the environmental conditions (Reil and Beutel 1976; Reil 1979; Sánchez-Zamora and Fernández-Escobar 2000), the phenological state of the tree (Reil 1979; Whiley et al. 1995; Sánchez-Zamora and Fernández-Escobar 2000), and tree health (Sachs et al. 1977; Lewis 1979).

Coniferous species are less effectively injected than angiosperms (Sachs et al. 1977; Reil 1979). The difference is explained by the wood structure. Conifer xylem is composed primarily of tracheids with greater resistance to water movement than angiosperms, in which the xylem contains large-diameter, vertical vessels. In addition, some coniferous species produce resin in response to tree wounds, which may affect water uptake. For these reasons, trunk injections in conifers are less frequent than in angiosperms, and less information is available on the factors affecting uptake and distribution of injected solutions in these species. The aim of the present study was to analyze some of these factors.

MATERIALS AND METHODS

The low-pressure injection system described by Navarro et al. (1992) was used in all experiments. The system uses a pressurized latex capsule, containing 250 mL (7.5 oz) of the solution to be injected, which is connected to a plastic injector that is inserted into a hole drilled into the tree trunk (Figure 1). Working pressure is between 60 and 80 kPa.

Figure 1. Injection capsules placed on tree trunk.

Influence of Injector Type, Tree Species, and Season on Uptake of Injected Solution

The experiments were performed on four resinous and three nonresinous species of conifers (Table 1). Within each species, trees with similar trunk diameters and leaf masses were used. For nonresinous species, six to ten trees (replicates) were selected per species. Three different injectors were used with a common length of 25 mm (1 in.) and diameters of 6, 4, or 3 mm (0.24, 0.16, or 0.12 in.). The 6 mm diameter injector is currently the most widely used. In each tree, three holes 50 mm (2 in.) deep were made, 6, 4, or 3 mm in diameter, at intervals around the trunk, and at heights ranging from 20 to 40 cm (8 to 16 in.) above the soil surface. The three injectors were then hammered into the wood to a depth of 15 mm (0.6 in.). Pressurized latex capsules containing 250 mL (7.5 oz) of water were then connected to the injectors.

The volume of water taken up at 1, 3, 24, and 48 hours after initiation was measured. Each capsule was divided into four equal sections and the following scale used to indicate the amount absorbed: 0 for 0%, 1 for < 25%, 2 for 25% to 49%, 3 for 50% to 74%, 4 for 75% to 99%, and 5 for 100%.

In resinous species (*Pinus* spp.), two injectors 70 mm (2.8 in.) long with diameters of 7 or 4 mm (0.28 or 0.16 in.), and the standard injector [6 mm (0.24 in.) in diameter by 25 mm (1 in.) long] were evaluated. Nine to ten trees (replications) were selected from each species. The procedure was as described for nonresinous species, with the exception that holes were drilled to a depth of 115 mm (4.6 in.) and the 70 mm (2.8 in.) long injectors were inserted to a depth of 60 mm (2.4 in.). Water uptake was measured at 24 and 48 hours after injection.

In these species, the experiment was conducted in March (end of winter rest) and in June (end of spring). Resin secretion was measured in ten trees for each species. For this purpose, two holes 65 mm (2.6 in.) deep and 6 mm (0.24 in.) in diameter were made in each tree on opposite sides of the trunk. Two 25 mm (1 in.) long injectors were inserted as described above, and a graduated 25 mL (0.75 oz) vessel was connected to each injector (Figure 2). Resin collected during the first 24 hours after the placement of the vessels was measured.

Distribution of Injected Rubidium in *Abies pinsapo*

Four healthy *Abies pinsapo* trees, with trunk diameters ranging from 16.2 to 19.5 cm (6.5 to 7.8 in.), were selected. Two holes 35 mm (1.4 in.) deep and 6 mm (0.24 in.) in diameter were made in each tree on opposite sides of the trunk between 10 to 20 cm (4 to 8 in.) above the soil surface. Injections were made as described above, using standard injectors [6 mm (0.24 in.) in diameter and 25 mm (1 in.) long] and pressurized capsules containing 250 mL (7.5 oz) of 50 mM rubidium chloride (RbCl) solution. Rubidium (Rb^+) was used as a distribution marker in leaves because it is a rare element in nature, moves through the symplast, is easy to extract from leaf tissues, and is widely used as a tracer for potassium (K^+). Solutions were completely taken up in less than 7 hours.

Needles from both current-season and previous-season growth were collected from either the northern or southern portion of each tree and from the basal, medium, or apical section of each tree 1, 2, and 12 days after injection. Thus, 12 different samples were taken at each time from each tree. Needles were collected in paper bags and stored in a portable ice chest. Once in the laboratory, needles were dried at 80°C (176°F) for 72 hours, ground, and stored in an oven at 60°C (140°F) until

Table 1. Coniferous species injected in the experiments.

Common names	Species	Average trunk diameter (cm/in.)
Resinous		
Stone pine	*Pinus pinea* L.	41.7 (16.7)
Cluster pine	*Pinus pinaster* Ait.	26.5 (10.6)
Aleppo pine	*Pinus halepensis* Mill	20.5 (8.2)
Canary Island pine	*Pinus canariensis* Sweet ex K. Spreng	18.7 (7.5)
Nonresinous		
Italian cypress	*Cupressus sempervirens* L.	19.5 (7.8)
Spanish fir	*Abies pinsapo* Boiss.	20.6 (8.2)
Deodar cedar	*Cedrus deodara* (D. Don) G. Don	28.3 (11.3)

Figure 2. Resin collection in a graduated vessel connected to an injector.

analysis. The stored samples were ashed in a muffle furnace at 600°C (1,112°F) for 12 hours and dissolved in 0.1 N HCl. Rb$^+$ content was determined using a Perkin Elmer 1100-B atomic absorption spectrophotometer.

In a second experiment, three *A. pinsapo* trees infected by the scolytus *Cryphalus abietis* Ratzeburg were selected to study the influence of health condition on movement and distribution of injected Rb$^+$. The same procedure described above was used, except that needles were sampled only from previous-season growth because of the shorter new growth caused by the insect, and the samples were collected from the basal and apical sections of each tree.

Statistical Analyses

Mean and standard errors were obtained for statistical comparisons. When the variable was qualitative, statistical analysis was based on mean values and the interval of variation between the data of each experiment.

RESULTS
Influence of Injector Size, Tree Species, and Season on Uptake of Injected Solution

Uptake volume varied among species and injector size, but it usually increased with time. In nonresinous species, the entire 250 mL (7.5 oz) was often taken up within 24 hours and, after 48 hours, most was completely taken up (Table 2). However, injection uptake was slower in resinous species and many solutions were only partially taken up within 48 hours. The most solution was injected into *Pinus pinaster* trees and the least into *P. pinea* trees.

In both resinous and nonresinous species, uptake volume usually decreased as injector diameter decreased. In nonresinous species, the 6 mm (0.24 in.) injector gave the best

results, but the 4 mm (0.16 in.) injector gave similar results in *A. pinsapo*. The 3 mm (0.12 in.) injector also gave acceptable results. In resinous species, the 7 mm (0.28 in.) injector was clearly the best, except in *P. canariensis* trees, which gave similar results to those obtained with the 6 mm injector.

Season also affected water uptake in resinous species (Table 3). Independent of injector size, less uptake occurred in June than in March. The exception was *P. canariensis,* in which higher uptake occurred in June if the 7 mm (0.28 in.) injector was used, and also *P. pinea* with the 6 and 4 mm (0.24 and 0.16 in.) injectors. Resin secretion in these species was less than 2 mL (0.06 oz) after 24 hours in March, but it increased in June, with significant differences among species (Figure 3). *Pinus canariensis* trees produced significantly less resin than the other species, followed by *P. pinea* trees. *Pinus pinaster* and *P. halepensis* secreted more than 20 mL (0.6 oz) of resin after 24 hours. These results may explain the low rate of water uptake when the trees were injected in June; at this time, the best results were obtained with *P. canariensis*.

Distribution of Rubidium in *Abies pinsapo*

Rubidium content increased over time in sampled needles (Figure 4). One day after injection, Rb$^+$ was recovered in all tree sections, indicating a homogeneous distribution throughout the tree. Rb$^+$ was subsequently redistributed in the tree, and higher contents were recovered in needles collected from the basal section of the tree than in samples collected from the upper part 12 days after treatment. Likewise, Rb$^+$ accumulated in higher quantities in younger than in the older needles (Figure 5). Significant differences were obtained on all dates, indicating that the accumulation in younger needles was before any retranslocation of Rb$^+$.

Uptake of RbCl was slow in trees infected by the scolytus *Cryphalus abietis*, with some trees resulting in less than 50% absorbed. Rb$^+$ was consistently lower in infected trees than in healthy trees (Figure 6). Rb$^+$ content in needles increased with time, although to a lesser extent than in healthy trees, and no significant differences were observed between Rb$^+$ content in needles collected from the basal and apical sections of the trees. However, Rb$^+$ was found in all sampled sections, indicating a homogeneous distribution also in these trees.

DISCUSSION

Coniferous species uptake solutions more slowly than angiosperms (Sánchez-Zamora and Fernández-Escobar 2000). In this study, however, 48 hours after injection of nonresinous species, all of 250 mL (7.5 oz) solution was taken up when the 6 mm (0.24 in.) injector was used. This rate of uptake is similar to that of angiosperms. Injection uptake in resinous species was incomplete in most of the cases. When these species were injected in spring, at the time of maximum resin secretion, water-injected uptake drastically decreased in all species, with the exception of

Table 2. Uptake rate of trunk injections in seven different coniferous species (resinous and nonresinous) during the first 48 hours following injections in relation to the injector size.

Species	No. trees	Month	Injector size (mm)	Uptake[*]							
				1 hour		3 hours		24 hours		48 hours	
				Mean	Range	Mean	Range	Mean	Range	Mean	Range
Nonresinous											
Cupressus	10	March	6			1.5	0–5	4.7	2–5	5.0	5–5
sempervirens			4			0.1	0–1	3.6	0–5	4.3	0–5
			3			0.0	0–0	3.6	0–5	4.4	0–5
Abies pinsapo	6	June	6	0.8	0–1	1.5	1–2	4.7	3–5	5.0	5–5
			4	0.7	0–1	1.0	1–1	4.5	3–5	5.0	5–5
			3	0.5	0–1	0.8	0–1	3.0	1–5	4.0	2–5
Cedrus deodara	7	August	6	0.0	0–0	1.4	1–2	5.0	5–5	5.0	5–5
			4	0.0	0–0	1.0	0–2	4.3	1–5	4.7	3–5
			3	0.0	0–0	0.7	0–1	2.7	1–5	4.2	2–5
Resinous											
Pinus pinea	10	March	7					2.8	0–5	2.8	0–5
			6					1.7	0–5	2.1	0–5
			4					0.3	0–3	0.5	0–5
Pinus pinaster	10	March	7					4.8	3–5	4.8	3–5
			6					2.9	0–5	3.2	0–5
			4					0.8	0–3	1.3	0–5
Pinus halepensis	10	March	7					3.7	0–5	3.7	0–5
			6					1.0	0–4	1.0	0–4
			4					0.4	0–3	0.4	0–3
Pinus canariensis	9	March	7					3.3	0–5	3.4	0–5
			6					3.3	0–5	3.3	0–4
			4					0.8	0–3	0.9	0–4

[*]Water volume uptake was measured on a scale 0 (0% water uptake) to 5 (100% water uptake). The table indicates mean and the minimum and maximum values within each species for each sample time.

Table 3. Uptake rate of trunk injections in four resinous coniferous species injected in winter (March) or in spring (June) in relation to the injector size.

Species	No. trees	Injector size (mm)	Uptake after 24 hours[*]			
			March		June	
			Mean	Range	Mean	Range
Pinus pinea	10	7	2.8	0–5	1.9	0–5
		6	1.7	0–5	2.5	0–5
		4	0.3	0–3	0.5	0–2
Pinus pinaster	10	7	4.8	3–5	0.3	0–1
		6	2.9	0–5	1.3	0–5
		4	0.8	0–3	0.1	0–1
Pinus halepensis	10	7	3.7	0–5	2.4	0–5
		6	1.0	0–4	0.8	0–1
		4	0.4	0–3	0.3	0–1
Pinus canariensis	9	7	3.3	0–5	4.4	1–5
		6	3.3	0–5	1.6	0–4
		4	0.8	0–3	0.8	0–3

[*]Water volume uptake was measured on a scale 0 (0% water uptake) to 5 (100% water uptake). The table indicates mean and the minimum and maximum values within each species for each sample time.

P. canariensis, which produced significantly less resin. These results suggest that the wood structure of conifers, composed primarily of tracheids, may explain the differences observed in solution uptake rates because they offer higher resistance to water movement but do not prevent the uptake of injected materials.

The poor uptake rate previously reported for conifers was obtained when *Pinus* spp. were injected at high pressure (Sachs et al. 1977; Reil 1979). The high-pressure systems usually need to be used in a short period of time on each tree because one tree must be finished before injecting the next. On the contrary, the low-pressure system used in these trials allowed the device to be placed in the trunk until complete uptake or at least, maximum uptake, had been achieved. These devices are independent, and the trees can therefore be injected independently of the other trees. This enables better results to be obtained with the low-pressure systems,

irrespective of the agreement of our results with the poor results reported earlier for *Pinus*. However, the poor uptake in resinous species could be attributed to the secretion of resin rather than to the anatomy of the wood, regardless of the injection system used.

Injector size also had a direct influence on solution uptake. In general, the uptake rates increased with increasing injector diameter. However, in nonresinous species, the 4 mm (0.16 in.) diameter injector gave acceptable results and works as well as the widely used 6 mm (0.24 in.) diameter injector in many cases, as has been observed also with many angiosperm species (Sánchez-Zamora and Fernández-Escobar 2000). The use of a narrower injector has the additional advantage of reduced healing time (Shigo 1979). The use of the 3 mm (0.12 in.) diameter injector must be limited to isolated cases because blockage of injections by shavings was observed in the drilled holes. In resinous species, the 4 mm diameter injector cannot be used. The greater length of the 7 mm (0.28 in.) diameter injector [70 vs. 25 mm (2.8 vs. 1 in.)] probably caused better uptake compared to the 6 mm diameter injector. The longer injector was used because it easily penetrates the thick bark of these species and reaches the xylem without harming the trunk.

Distribution of injected materials throughout the tree is an important factor that may limit the use of the technique because chemicals may accumulate in one part of the tree and not in others. Many factors, including hole depth, injection placement, tree structure, and the number of injections per tree, affect the distribution of solutions (Sachs et al. 1977; Navarro et al. 1992). The injection method also affects distribution. Today, the tendency is to use low-pressure systems comprising individual devices on each injection point in order to control the quantity of material applied on each point (Whiley et al. 1991; McClure 1992; Navarro et al. 1992). Most of the studies on distribution have been developed in angiosperms, and little is known about distribution in conifers. In the present study, distribution of injected Rb+ in *A. pinsapo* was similar to that observed in olive trees (Navarro et al. 1992), with a tendency for accumulation in the basal area of the trees and in younger needles. But Rb+ distribution in *A. pinsapo* was more homogeneous than in olives, and Rb+ content in needles was more than twice that obtained in olive leaves when the same quantities were injected.

The state of health also influences uptake of injected solutions, as reported elsewhere (Sachs et al. 1977). Unhealthy fir trees were more difficult to inject because both uptake rate and Rb+ content in needles were lower than in healthy trees. However, Rb+ was effectively distrib-
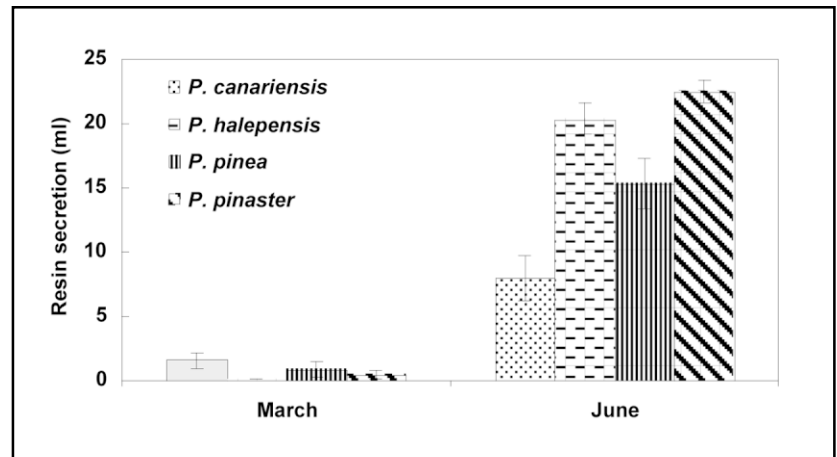

Figure 3. Resin secretion of four resinous species 24 hours after the treatment. Vertical bars indicate the SE.

uted in all cases, indicating that uptake of injections is usually slower in conifers than in angiosperms, although solutions are effectively distributed throughout the tree. This may explain why in trials in which winter injections of acephate were used to control the Mediterranean pine processionary caterpillar (*Thaumetopoea pityocampa* Schiff), the insect was efficiently controlled even when the injections were only partially taken up (Fernández de Cordova and Gallego 1995).

The coniferous species tested can be injected efficiently with aqueous solutions. Their wood structure makes uptake of injected solutions slow, but the distribution throughout the tree is usually more uniform than in angiosperms. Injection was more difficult in resinous species, particularly in spring when there is more resin secretion. In terms of simplicity and labor cost, as well as the slowness of uptake, the low-pressure method of application used here efficiently injects coniferous trees. The injector could be reduced in size to 4 mm (0.16 in.) in diameter for use in nonresinous species, but length must be increased to 70 mm (2.8 in.) to inject trees with thick bark and to reach the xylem without damaging the trunk.

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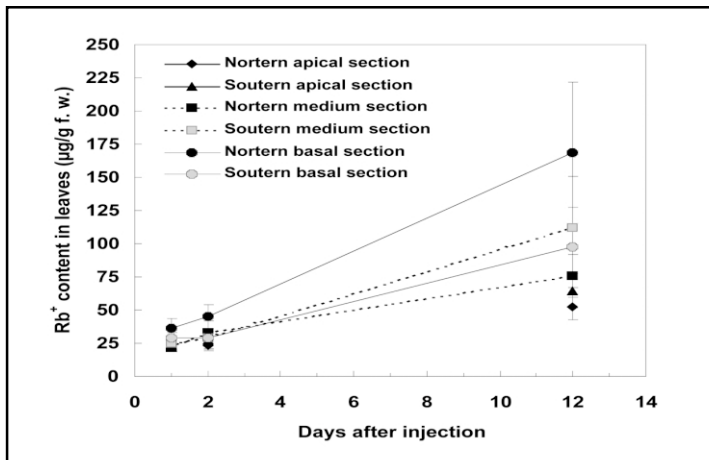
Figure 4. Rubidium content in needles of *Abies pinsapo* collected from six different sections of the trees. Vertical bars indicate the SE.
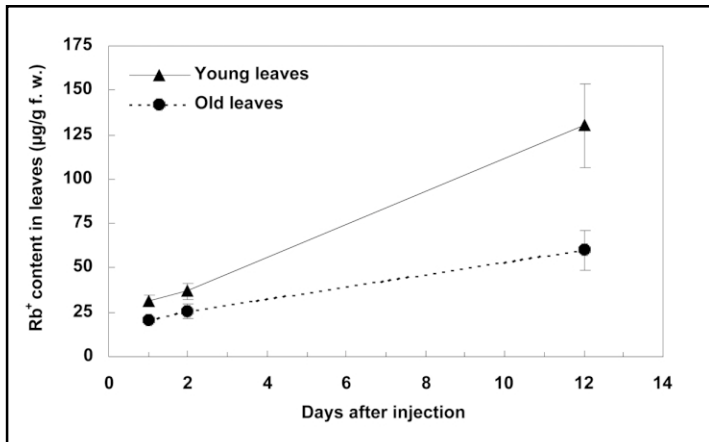
Figure 5. Rubidium content in current-season (young) and previous-season needles (old) of *Abies pinsapo*. Vertical bars indicate the SE.
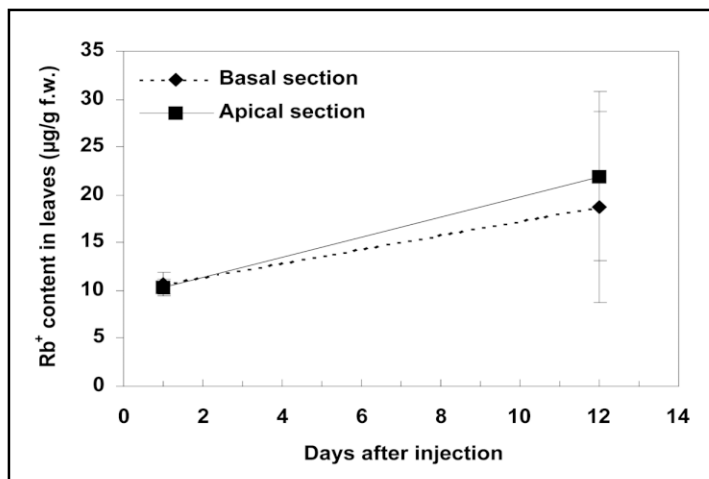
Figure 6. Rubidium content in needles collected from the apical and basal sections of *Abies pinsapo* trees infected by the scolytus *Cryphalus abietis*. Vertical bars indicate the SE.

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Résumé. Sept espèces de conifères, dont quatre résineuses, ont été injectées avec des capsules de latex pressurisées contenant 250 ml d'eau au moyen de différentes dimensions d'injecteurs allant de 3 à 7 mm de diamètre et de 25 à 70 mm de longueur. Dans les espèces résineuses, l'absorption était lente, souvent incomplète, particulièrement au printemps lorsque les sécrétions de résine étaient élevées. Le taux d'assimilation de l'eau diminuait avec l'emploi des injecteurs aux diamètres les plus petits. Le taux d'assimilation de l'eau le plus élevé se faisait avec l'injecteur de 7 mm de diamètre et de 70 mm de longueur. Chez les espèces non résineuses, l'injecteur de 4 mm de diamètre fonctionnait aussi bien que celui plus communément utilisé de 6 mm de diamètre dans la plupart des cas, et ce avec l'avantage supplémentaire de la réduction du diamètre de la blessure et d'une diminution de la période de temps nécessaire pour la cicatrisation. Des sapins d'Espagne ont été injectés avec une solution de 50 mM de chlorure de rubidium. Le Rb+ a été retrouvé dans toutes les sections d'arbres échantillonnées, autant ceux en bonne qu'en mauvaise santé. Les arbres en santé avaient un taux et une vitesse d'assimilation plus élevés que ceux non en santé. Les résultats indiquent que les conifères ont été injectés de manière efficace. Le taux d'assimilation est plus faible que chez les angiospermes mais la distribution est plus uniforme. En raison de cette lenteur, les méthodes d'injection par faible pression dans le tronc qui se composent de pièces individuelles au niveau de chaque point d'injection sont plus efficaces pour injecter les espèces de conifères.

Zusammenfassung. Sieben Koniferenarten, vier davon harzend, wurden injiziert mit unter Druck stehenden Latexkapseln, die 250 ml Wasser enthielten, mit der Hilfe von vier verschiedenen Einspritzgrößen von 3–4 mm Durchmesser und 25–70 mm Länge. In den harzenden Arten war die Aufnahme langsam und manchmal nicht vollständig, besonders im Frühling, wenn die Harzproduktion anstieg. Die Wasseraufnahmerate sank, als die kleineren Spritzen benutzt wurden. Eine 7 mm Kanüle mit einer Länge von 70 mm führte zur schnellsten Wasseraufnahme. Bei den nichtharzenden Arten funktionierte der 4 mm Injektor genau so gut die die allgemein verwendeten 6 mm Injektoren mit dem zusätzlichen Vorteil von reduzierter Wundgröße und verkürzter Heilungszeit. Spanische Tannen wurden mit einer 50mM-Lösung aus Rubidiumchlorid injiziert. Rb+ wurde in allen Proben bei gesunden und kranken Bäumen gefunden. Gesunde Bäume hatten eine größere und schnellere Aufnahme als Kranke. Die Ergebnisse weisen darauf hin, dass Koniferen effizient gespritzt wurden. Die Aufnahmerate in Angiospermia ist geringer, aber die Verteilung ist gleichmäßiger. Wegen dieser Langsamkeit sind die Niedrigdruckeinspritzmethoden unter Verwendung von individuellen Vorrichtungen an jedem Injektionspunkt für die Behandlung von Koniferen effektiver.

Resumen. Siete especies de coníferas, cuatro de ellas resiníferas, fueron inyectadas con cápsulas de látex presurizadas con capacidad de 250 ml de agua, empleando diferentes tamaños de inyector entre 3 y 7 mm en diámetro y de 25 a 70 mm de longitud. En las especies resiníferas la absorción fue más lenta y algunas veces incompleta, particularmente en primavera cuando la secreción de resina fue más alta. La tasa de absorción de agua disminuyó cuando se emplearon inyectores de menor diámetro. El inyector de 7 mm de diámetro y 70 mm de longitud fue el que logró las absorciones más rápidas. En las especies no resiníferas el inyector de 4 mm de diámetro trabajó en muchos casos tan bien como el más ampliamente usado de 6 mm, con la ventaja de reducir el tamaño de la herida y disminuir el tiempo de cierre de la misma. Se inyectaron abetos españoles con 50 ml de una solución de cloruro de rubidio. El Rb+ fue recubierto en todas las secciones de muestra del árbol tanto para árboles saludables como no saludables. Los árboles saludables tuvieron una mayor y rápida tasa de absorción que los no saludables. Los resultados indican que las coníferas fueron inyectadas eficientemente. La tasa de absorción es más lenta en las angiospermas pero la distribución es más uniforme. Debido a la lentitud, los métodos de inyección al tronco a baja presión, compuestos de aparatos individuales en cada punto de inyección, son más eficientes para la inyección de árboles de coníferas.