Trunk Injection Method for Introducing Chemical Formulations into *Hevea brasiliensis* in View of Controlling White Root Disease

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ABSTRACT

Rubber (*Hevea brasiliensis*) is an important plantation crop in Sri Lanka. One of the major setbacks of the rubber industry is diseases. White root disease has become the most destructive root disease of rubber. An experiment was conducted to improve the novel application technique, tree injection in view of controlling white root disease. Forty rubber trees of the clone RRIC 121 was used to test the effect of injection angles of 45 and 60 degrees to the horizontal and two depths of 4 and 1.5 cm from the outer bark on uptake rates of injected tebuconazole (Folicur,250 EC, Bayer) solutions. The distribution of the injected solutions was tested with the tracer dye, Rhodomine B using eight years' old budwood nursery plants. The uptake volumes obtained 48 hrs after injection were statistically insignificant for the two angles and two depths. There was no interaction effect evident. The dye distributed at all the angles in roots and in shoots but failed to consistently deliver when injected at the depth of 4 cm. Injection angle of 45 degrees to the horizontal is the most practicable angle and to effectively distribute the dye in the outer regions of the plant, both roots and shoots, injection at a depth of 1.5 cm is recommended. However, based on the observations from this study, tree injection method is showing promising results towards cost effective and environmental friendly chemical control of white root disease.

KEYWORDS: *Hevea brasiliensis*, Tree injection, Uptake time, White root disease

INTRODUCTION

Natural rubber plantation industry plays a major role in Sri Lankan economy as a plantation crop. The rubber extent presently in Sri Lanka is around 122,240 ha (Silva, 2010). It is an important tree crop as it produces latex with commercial value. Rubber provides a range of benefits to the Sri Lankan economy by generating job opportunities, making export earnings and providing livelihood of thousands of people. In terms of ecological benefits, rubber contributes thousands of hectares as a forest crop that ultimately results in environmental sustainability.

Rubber plantations are affected by several economically important diseases. These diseases can be divided for convenience into four categories: leaf; stem and branch, panel and root diseases. Among them, white root disease is very serious in many rubber growing countries (Jayasinghe, 2010). White root disease is caused by the fungus *Rigidoporus microporus.* The fungus penetrates the roots and causes damage to the water conducting tissues and as such the first above ground symptom is the general discoloration of the foliage. Based on a survey conducted, land area infected with white root disease is approximately between 5-10 % in Sri Lanka (Liyanage, 1977). In the wet zone of Sri Lanka the rubber cultivations are now in their fourth generation. As a result, the potential of buildup of the white root disease inoculums and the alternate host trees have become naturally higher (Fernando *et al.*, 2012). White root disease can be controlled by drenching systemic fungicides such as tebuconazole or hexaconazole (Silva, 2010). But drenching of fungicides creates environmental concerns. So trunk injection can be used as a more efficient chemical application technology.

Tree injection has been practiced since as early as the 12th century (Zamora and Escobar, 2004). Later, Leonardo da Vinci was, apparently, the first person who developed systematic experiments with liquid injection into trees (Zamora and Escobar, 2004). Trunk injection requires low volumes of chemicals, thus resulting in reduced cost in controlling the disease. Minimum wastage of chemicals could be achieved together with reducing the impact of fungicides on the environment compared to other conventional chemical application method such as soil drenching. In this background, this study aimed to investigate the possibility of the new application technique, trunk injection for *Hevea brasiliensis* in view of controlling white root disease.

MATERIALS AND METHODS

Location

This study was conducted at the Plant Pathology and Microbiology Department of the Rubber Research Institute of Sri Lanka, Dartonfield, Agalawatta from January to April 2013.

Injectors

The pressurized injection system described by Guest *et al.*, (1994) was used in all experiments. The Chemjet injectors (Chemjet Trading (Pvt) Ltd.) which contain 20 ml of the solution and have a working pressure of 1 to 1.5 bars were used to introduce chemicals into trees through drilled holes.

Effect of Injection Angle and Depth on Uptake of Injected Solutions

The experiments were carried out on healthy mature rubber trees of the clone RRIC 121 at Dartonfield Estate, Rubber Research Institute of Sri Lanka (RRISL), where the trees had an average trunk circumference of 89 cm at 40 cm above soil surface. Experiment was laid out in a two factor factorial design. Forty trees were selected and numbered using paint and each tree was drilled using a battery driven driller (Bosch, GSR 12-20) which contained a drill bit of diameter of 4 mm. Three holes per tree were made at two angles of 45 and 60 degrees to the horizontal on a clear day. The trees were drilled to a depth of 4 cm from the outer bark. The holes were made at regular intervals around the trunk, and at heights ranging from 10 to 40 cm above the soil surface. Drilling commenced at 9 a.m and the trees were left 4-5 hrs until the latex flow had stopped. Then the drilled holes were cleaned using a metal wire which was used to remove the coagulated latex inside holes. Meanwhile aqueous solution of tebuconazole (Folicur, 250 EC, Bayer) was prepared at a concentration of 12% and 120 injectors were filled with the solution. Then the injectors were inserted into drilled holes at around 4 p.m on the same day. The amount of solution taken up by the trees was measured at 24 and 48 hrs after injection. The same procedure was repeated on another day where the holes were drilled at 45 and 60 degree angles at a depth of 1.5 cm from the outer bark. The same group of 40 trees were used again with changing the hole position. The volumes taken up by the plants were measured using a scale prepared according to the length of the injector.

Distribution of Injected Solutions

Distribution of injected solutions within the plant was studied using eight years old budwood nursery plants of the clone RRIC 121 at Dartonfield Estate, RRISL where the plants had an average trunk circumference of 36 cm at 15 cm above soil surface. Nine budwood nursery plants were selected to evaluate the injection angles of 0, 45 and 60 degrees to the horizontal at a depth of 2.5 cm. Three plants per each angle were used. Two holes per tree were drilled at a height of 15 cm above soil surface. In order to observe the injected liquid, injections were made as described above using the tracer dye, Rhodomine B at a concentration of 2% in an aqueous solution. The nine plants were uprooted one week after injection to observe the distribution of the dye both upwards and downwards within the plant. To visualize the dye, outer bark of roots, stems and shoots was removed using a knife.

Meanwhile another six budwood nursery plants were used to evaluate the injection depth on the distribution of the dye. Two depths of 1.5 cm and 4 cm were tested using three plants per each depth. One week after the injection, all the plants were uprooted and the dye content was observed as mentioned above.

Statistical Analysis

Statistical analysis of mean values was performed by general linear model (GLM) using statistical software Minitab Version 15.

RESULTS

Effect of Injection Angle and Depth on Uptake of Injected Solutions

Uptake of tebuconazole was slightly higher in deep injected method compared to shallow injection method (Table 1), but the volumes obtained were not significantly different (Table 2). The uptake volumes obtained for the two angles were also statistically insignificant. Also there was no interaction between injection angle and the depth on uptake of injected solutions. The entire 20 ml of the injected solution was completely taken up by more than 70% of the trees within 48 hours after injection. Trunk diameter had no consistent effect on the uptake of injected solutions (Figure 1). However the uptake volumes increased with time (Figure 2).

Table 1. Uptake volumes (ml) obtained, 48 hrs after injection by changing injection angle and depth

	Angle	
Depth	**45^0**	**60^0**
Deep (4 cm)	14.46	14.73
Shallow (1.5 cm)	13.27	12.91

Table 2. Effect of different treatments on the uptake rate

Source	DF	Mean square	F	P
Depth	1	45.53	2.55	0.114
Angle	1	0.04	0.00	0.964
Depth * Angle	1	0.11	0.11	0.739

Significantly different at 0.05 level

Distribution of Injected Solutions

The tracer dye Rhodomine B was evident in roots and in shoots when injected at all three angles of 0, 45 and 60 degrees. The dye distributed downwards throughout the tap root and was also evident even in lateral roots of pencil thickness size. The dye distributed approximately 80 cm from the injection point downwards and even distributed 3 m upwards when the branches were examined.

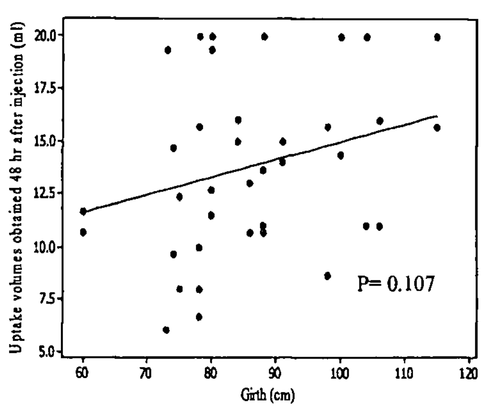

Figure 1. Relationship between girth and uptake volumes 48 hrs after injection

In relation to depth, the dye did not distribute to the end of the tap root in all trees when deep injected. Upward movement was also not evident in all trees. However in shallow injected trees, the dye moved downwards throughout the tap root in all trees. Upward movement was also very much evident in all trees. In shallow injected trees the dye was evident just after removing the outer bark, where as in deep injected trees, dye was detected after removing large portion from the outer region.

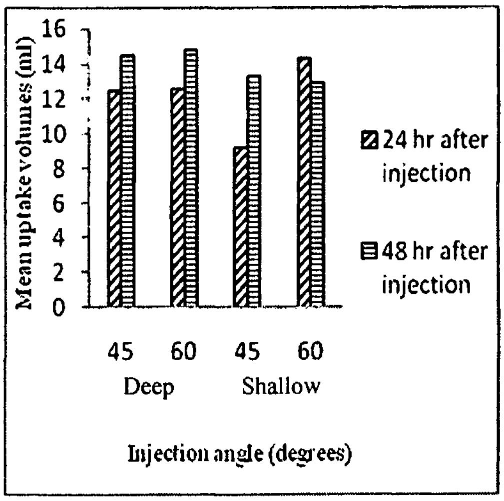

Figure 2. Uptake volumes obtained for different treatment combinations

DISCUSSION

Results obtained in this study prove the fact that trunk injection could be applied for rubber trees. With the added advantage of rubber being a crop which is not utilized as a source of food, introduction of fungicides into trees will not be an issue. As the injection angles were insignificant in relation to uptake rates obtained, injection angle of 45 degrees to the horizontal could be recommended considering the practical aspects. Injection angle of 60 degrees to the horizontal was difficult to adopt at field level as it was difficult to drill holes because of damages to the outer bark. It was easy to drill and insert injectors at 45 degree angle. When injections were conducted parallel to the ground level (0 degrees), it was unsuccessful because of chemicals inside injectors leaked through the handle. So 45 degree angle is the best as recommended for coconut palms against the red weevil attack (Anon, 2006).

Experiments conducted using the tracer dye, Rhodomine B showed very positive results, as the dye was evident in roots at all three injection angles of 0, 45 and 60 degrees. The movement of the dye upwards could be through the xylem tissue driven by transpiration. However the downward movement of the dye against the transpiration stream is an unusual occurrence where it questions the mechanism of movement of solutions through xylem vessels. The downward movements may occur through xylem due to the high molecular weight of Rhodomine B (479.01 g / mol) or it may be due to high pressure applied by the chemjet injectors (1-1.5 Bars). Shallow injections distributed the dye throughout the tap root and small roots where as deep injections failed to do this consistently. The dye distributed downwards along the sapwood or the active xylem vessels when shallow injected. To suppress the fungus *Rigidiporus microporus* which causes white root disease, fungicides have to be distributed downwards through active xylem vessels. Based on these observations shallow injection could be recommended in view of controlling white root disease. Deep injected dye solutions moved downwards mostly through inactive xylem vessels (heartwood). This occurrence is insignificant in controlling the disease. Hence shallow injected fungicides resulted in translocation them to the finest root tips. This will reduce the spread of the disease to surrounding healthy plants. However to confirm the movement of the injected fungicide tebuconazole throughout the tree, chemical analysis of twig, leaf and root samples is needed to determine the concentration of fungicide in areas distant from injection sites.

To effectively practice tree injection for *Hevea brasiliensis,* there must be at least 4-5 hrs time gap between drilling holes and inserting injectors. The coagulated latex inside holes has to be removed; if not tips of the injectors will be blocked which results in failure of uptake of chemicals injected. This was evident after removing injectors which did not perform well. Further studies must be conducted to evaluate other factors affecting uptake and distribution of injected solutions. Moreover the behavior of different chemicals, their efficiency should also be evaluated under field conditions to make firm conclusions of their practical ability to suppress plant diseases. If an efficient method is developed to inject chemicals targeting the active phloem region in trees, white root disease could be effectively controlled.

CONCLUSIONS

The results revealed that trunk injection for *Hevea brasiliensis* was efficient at an angle of 45 degrees to the horizontal both in terms of uptake & practicality. A depth of 1.5 cm from the outer bark was found to be effective to distribute the dye to the outer regions of the plant, both roots and shoots. Therefore, as evident from this study shallow injection method may be used to provide fungicides to finest root tips.

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