LABORATORY AND GREENHOUSE EVALUATION OF SOME SOIL FUMIGANTS FOR TOXICITY AGAINST FIVE ROOT PATHOGENS OF TEA

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The effectiveness of nine soil fumigants against five root rot pathogens of tea was evaluated in the laboratory using three different techniques. The techniques employed were effective in providing information on (a) the innate volatile toxicity, (b) capacity to penetrate and kill the pathogen within host tissue, and (c) activity in the soil, of each of the nine soil fumigants. The ability of the chemicals to kill the pathogen within host tissue and to move through the soil and kill the fungus in agar inoculum was directly related to their volatile toxicity. There was good correlation between the laboratory tests and pot tests conducted in the greenhouse, and a promising material, viz WN 12, was detected in these studies.

There was also a highly significant interaction between fungicides and fungi indicating that some fungicides may be highly specific in their action. It was evident that for a rapid screening test to select the most promising soil fumigant, one of the techniques described (Method III) would be sufficient, if infected root segment is substituted for agar disc inoculum. Such an assay would select compounds that penetrated soil and plant tissue and toxic to the test fungus.

For many years the root rots of tea were controlled by the laborious and expensive digging and removal of infected roots. The efficacy of this practice varied. Recently, soil fumigation with chemicals like D-D and methylbromide has shown better results, especially in the control of Red Root Disease, the most important of the root rots (Shanmuganathan 1964 ; Shanmuganathan and Redlich 1965). This aroused interest in the chemical control of tea root diseases and nine potentially effective soil fumigants were tested in the laboratory for effectiveness against five root pathogens, using D-D as the standard material. These investigations are described in this paper.

Several assay techniques have been developed in the past to evaluate soil fungicides in the laboratory. On the whole, many of these techniques served to study only one aspect of the problem, such as inhibition of spore germination or of mycelial growth on nutrient media or soil, volatile toxicity in closed chambers, etc, and seldom have these been integrated to give a complete picture of the individual compounds. Recently Corden and Young (1962) employed three separate techniques to study respectively fungitoxicity, drenchability, and capacity to kill fungi within plant tissues of 19 fungicides, and they concluded that the ability of the different fungicides to move through the soil was associated principally with their volatility. In this study, three similar techniques were used to determine separately : a) the innate volatile toxicity or effectiveness in the vapour phase, b) the capacity to penetrate and kill the pathogen within host tissue, and c) effectiveness in the soil when applied as a drench to the soil surface.

Materials and methods

Method 1. *Innate volatile toxicity or effectiveness in the vapour phase.*—Innate volatile toxicity was assessed on the basis of the degree of inhibition of radial growth of the fungus in culture plates using the technique described by Latham and Linn (1965).

A 30 mm diameter glass dish was placed inside a 120 mm Petri dish with cover and sterilized in an oven. The inside dish was centred and 35 ml of sterilized potato-dextrose agar (PDA) was poured into the annular space.

The test fungi were grown on PDA for 10 days at 25°C and two inoculum plugs, 7 mm in diameter and 3 mm thick, were cut with a sterile cork-borer from the periphery of the colony of each fungus. The plugs were placed with the mycelium side down on opposite sides of the annular space (Fig. 1). One ml of fumigant was suspended in 1 litre of distilled water and a ml of this suspension (1000 ppm) was pipetted into the inner dish. The top of the bottom Petri dish was coated thoroughly with silicone grease and the cover was then pressed on to form an air-tight chamber. There were three replicates for each fumigant, and three check plates contained only distilled water in the inner dish. The silicone grease used had slight fungistatic effect on the test fungi but this was disregarded. The plates were incubated at 25°C and the diameter of the colonies was measured after 7 days.

Method II. *Penetration into plant tissue.* — For experiments on penetration into root tissue, Roux culture tubes were used. These are large test tubes about 8 in. long and 1 in. in diameter and with an indentation 2 in. from the base, which served to prevent direct contact between fungicide and inoculum. Four ml of the fungicide were carefully pipetted into the well of the tube and a piece of infected root was placed inside the tube ; the tube was then closed tightly with a rubber stopper and incubated at room temperature (20-22°C).

Inoculum was prepared by inoculating segments of tea roots, 1.5 in. long and 0.5 in. in diameter, with the test fungi and incubating them for three months.

After 15 min. of exposure to the fumigant, the inoculum was removed, split into four and cultured on PDA to determine the viability of the test fungus (Fig. 2). This procedure was repeated with inccula treated for 30 min., 1, 2, 4, 8, 16 and 24 hr, each treatment being replicated five-fold.

Method III. *Effectiveness in soil-tubes.* — This assay method using non-sterile soil served to evaluate the true fungicidal activity in the soil and was particularly intended to measure the movement of the fumigants in the soil following their application to the soil surface.

The technique adopted was a modified form of Zentmyer's method (1955). The soil used was a clayey-loam of pH 5.5, collected from a tea field near the laboratory at St Coombs. After air-drying and sifting through a 100-mesh sieve, 100 g of the soil held 58 g of water. 225 g of this soil was packed in a large boiling tube to form a column 9 in. high and 1.5 in. in diameter, and 60 ml of water was added to bring the moisture content to approximately 50% field capacity. During the packing, 9 discs of inoculum, 18 mm in diameter and 3 mm in thickness, were placed one in. apart in the soil column. The top disc was one in. below the soil surface. The inoculum discs were taken from the periphery of 10-day-old cultures of the test fungi on PDA.

0.1 ml of the fumigant was pipetted onto the soil surface, the tubes stoppered and incubated at room temperature for 24 hr. At the end of this period, the discs were carefully removed from the soil, washed well and placed in order on moist filter paper in a Petri dish, and incubated at room temperature to determine the viability of the test fungus (Fig. 3).

Pot experiment in the greenhouse. — In this experiment, seven fumigants were tested for effectiveness in controlling *P. hypolateritia* in pots.

10-inch pots were filled with tea soil and an infected root segment, 4 in. long and 1 in. in diameter, and prepared as previously described, was buried in each pot about 3 in. below the surface. 1, 2, 4, 8 or 16 ml of fumigant was then applied to each pot and the pots lightly watered and covered with chopped Guatemala grass

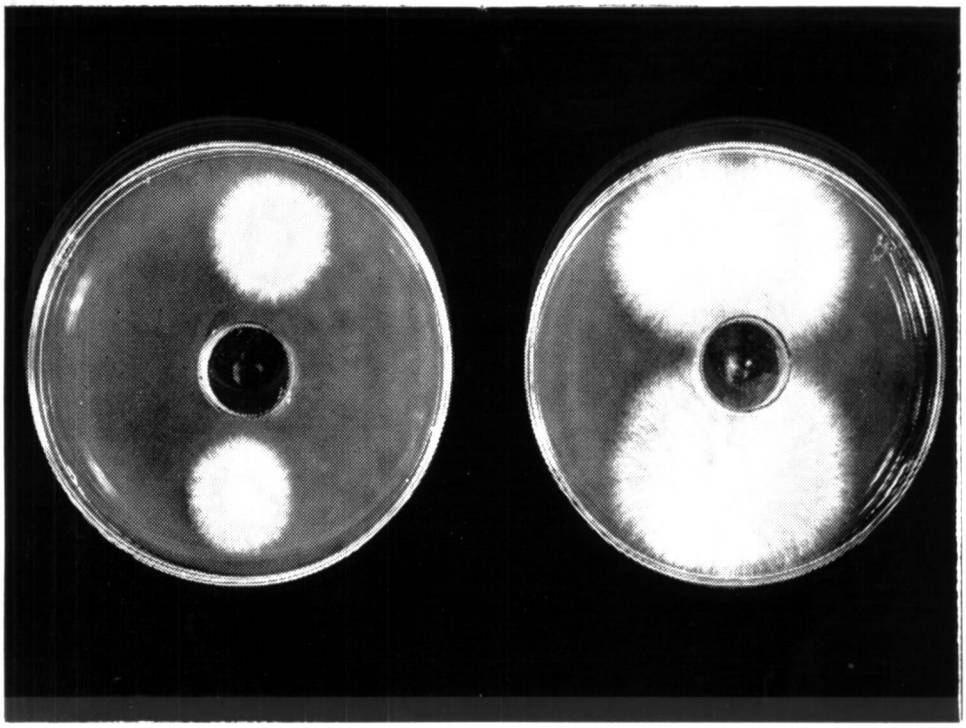

FIGURE 1—*Growth of* Poria hypolateritia *after exposure to different soil fungicides for 7 days in 'Petri dish' tests*

FIGURE 2—*Viability of* Poria hypolateritia *after exposure to different soil fungicides for various times*

Left — control; Right — treated

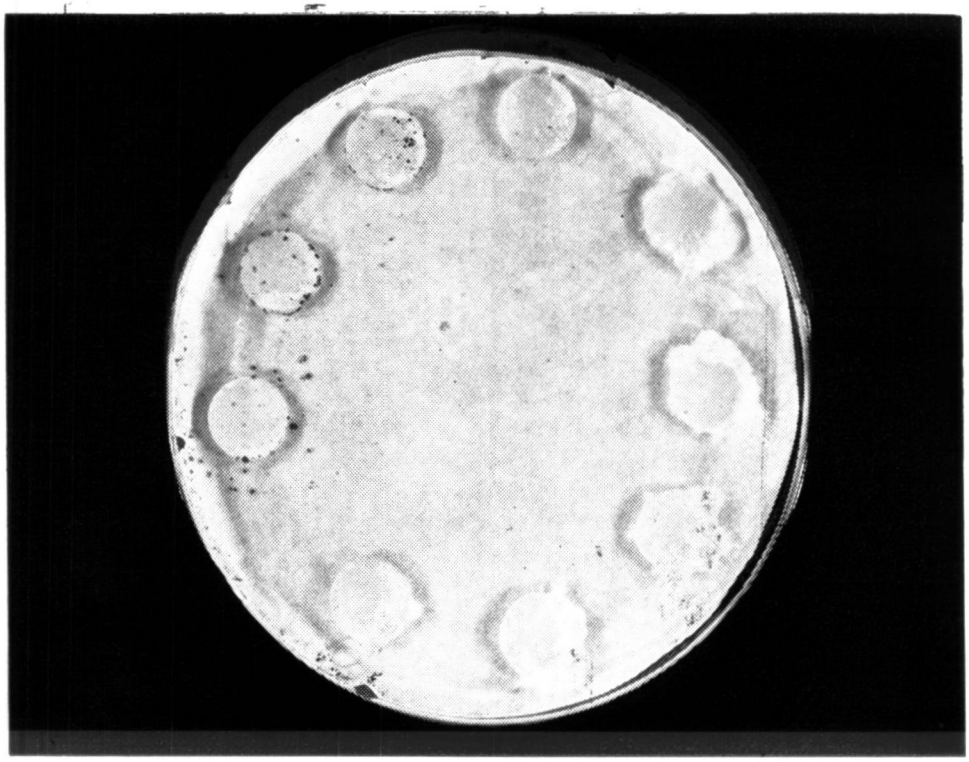

FIGURE 3—*Viability of agar disc inocula containing* Poria hypolateritia *after exposure to different soil fungicides for* 24 *hours. Note absence of* P. hypolateritia *mycelium and presence of* Trichoderma viride *on the* 4 *discs on top, left hand side*

leaves. There were five replicates for each concentration and five untreated pots served as controls. Three months after treatment, the root segments were removed and incubated in a moist chamber to determine the viability of the test fungus.

Materials — The chemicals tested and their active ingredients are given in Table 1.

TABLE 1.—*Chemicals tested for toxicity to five root rot fungi*

Common designation	Active ingredient
D–D	1,3–dichloropropene and 1,2–dichloropropane
Vapam	Sodium n–methyl dithiocarbamate 31%
Trapex	Methyl isothiocyanate 20%
WN12	Methyl isothiocyanate 20% and 1,3–dichloropropene and related compounds 80%
Dowfume W–85	Ethylene dibromide 83%
Formalin	Formaldehyde 40%
Nemagon	1,2–dibromo–3–chloropropane 95% and other halogenated C_3 compounds 5%
Fumazone 70 E	1,2–dibromo–3–chloropropane 67.5% and other halogenated C_3 compounds 3.5%
Carbon disulphide	Carbon disulphide

TABLE 2 — *Toxicity of nine soil fumigants to five root pathogens of tea in Petri dish tests*

Fungus	Mean diameter of colony (mm)										
	Fungicide										Significant difference
	WN 12	Vapam	Trapex	DD	Nemagon	Formalin	Fumazone	Carbon disulphide	Dowfume	Control	($P < 0.05$)
Poria hypolateritia	25.0	20.7	22.7	31.7	44.7	43.2	43.8	48.3	47.2	61.0	11.1
Fomes noxius	23.8	34.3	54.2	51.2	44.3	71.2	70.3	80.4	79.5	76.0	6.7
Fomes lignosus	25.2	17.2	47.2	61.3	65.2	69.2	69.8	74.5	73.3	71.2	6.3
Ustulina deusta	0	27.8	30.3	41.8	41.8	48.7	49.7	50.0	49.3	50.1	3.5
Rosellinia arcuata	29.0	35.5	42.6	54.3	54.6	53.2	57.2	54.8	57.6	60.7	4.9
Mean	20.3	27.1	39.3	50.0	50.0	56.7	58.2	61.4	61.5	63.9	2.3

Test fungi — The fungi selected for study are given below, together with the diseases for which they are responsible.

Poria hypolateritia Berk	Red Root Disease
Fomes noxius Corner	Brown Root Disease
Fomes lignosus Klotzsch	White Root Disease
Ustulina deusta (Fr.) Petrak	Charcoal Stump Rot
Rosellinia arcuata Petch	Black Root Disease

Results

Innate volatile toxicity — The five fungi selected for study were tested separately, since 50 plates were about the maximum that could be conveniently handled in one trial (i. e. 9 fungicides + control × 5 = 50). Accordingly, a test was first made with *P. hypolateritia*, and then repeated with each of the other four fungi.

The results of the five trials are summarized in Table 2. It will be seen that none of the nine fumigants tested was effective in completely inhibiting the growth of any of the five fungi, except WN 12 which was lethal to *U. deusta*. In the case of *P. hypolateritia*, all fungicides caused significant reduction in growth compared with the control, and the nine compounds fell into two groups in regard to their effectiveness. The first group consisting of WN 12, Vapam, Trapex and D-D was significantly superior to the second group, which was made up of Nemagon, formalin, carbon disulphide, Dowfume and Fumazone. In contrast to *P. hypolateritia*, only five fungicides, viz WN 12, Trapex, Vapam, D-D and Nemagon, were effective against *F. noxius ;* formalin, Fumazone, carbon disulphide and Dowfume had no significant effect on its growth. The effectiveness of the chemicals against *F. lignosus* was essentially similar to that of *F. noxius* but with the exception that Nemagon had also no significant effect on its growth. WN 12, Vapam, Trapex, D-D, Nemagon and formalin caused significant reduction in the growth of *R. arcuata*, the first three compounds being more effective than the last three.

When the activity of all the nine compounds is considered as a whole against the five fungi, it can be seen that WN 12, Vapam and Trapex are much superior to D-D, which is of the same order of effectiveness as Nemagon and formalin. The vapour phase activity of Fumazone, carbon disulphide and Dowfume appears to be of a very low order. Of the three most effective materials, WN 12 and Vapam are significantly better than Trapex.

A highly significant interaction ($P < 0.001$) between fungicides and fungi was also evident in these trials and the implications of this will be discussed later.

Of the five test fungi, *P. hypolateritia* and *U. deusta* appeard to be the most sensitive to the nine fungicides, while the two *Fomes* spp. were the most resistant (Table 3).

TABLE 3 — *Susceptibility of five fungal pathogens of tea to nine soil fumigants in Petri dish tests*

Mean diameter of colony (mm)				
Poria hypolateritia	*Ustulina deusta*	*Rosellinia arcuata*	*Fomes lignosus*	*Fomes noxius*
38.8	39.0	50.1	57.2	59.2

Significant difference ($P < 0.05$) = 2.3

TABLE 4 — *Toxicity of nine soil fumigants to five root pathogens of tea in root sections*

Fungus	Mean No. of root segments out of five showing viable fungus (transformed data)										Significnat difference
	Fungicide										
	WN 12	Trapex	Vapam	DD	Carbon disulphide	Formalin	Nemagon	Dowfume	Fumazone	Control	($P < 0.05$)
Poria hypolateritia	1.18	1.05	1.70	1.88	2.15	2.40	2.09	2.40	2.45	2.45	0.34
Fomes noxius	1.49	1.55	1.40	1.91	1.90	2.27	2.27	2.45	2.42	2.45	0.40
Fomes lignosus	1.10	1.10	1.52	1.91	2.03	2.19	2.42	2.45	2.27	2.45	0.38
Ustulina deusta	1.09	1.09	1.85	2.00	1.98	2.12	2.45	2.23	2.39	2.45	0.33
Rosellinia arcuata	1.10	1.37	1.93	2.00	2.63	2.31	2.39	2.34	2.40	2.45	0.35
Mean	1.19	1.23	1.67	1.94	2.02	2.26	2.32	2.37	2.39	2.45	0.20

TABLE 5 — *Toxicity of nine soil fumigants to four root pathogens of tea in soil-tube tests*

Fungus	Mean No. of agar discs out of nine showing viable fungus: Fungicide										Significant difference
	WN 12	Vapam	Trapex	DD	Nemagon	Dowfume	Carbon disulphide	Fumazone	Formalin	Control	($P < 0.05$)
Poria hypolateritia	3.7	4.0	2.7	6.0	7.7	7.3	7.7	7.3	7.7	9.0	1.1
Fomes lignosus	0.7	3.3	3.7	4.7	6.0	8.7	7.7	9.0	8.0	9.0	0.8
Ustulina deusta	1.7	3.3	4.0	5.0	4.7	3.3	7.0	6.7	8.0	9.0	0.9
Rosellinia arcuata	1.0	3.3	5.3	3.3	4.0	5.3	7.0	9.0	8.7	9.0	1.7
Mean	1.8	3.5	3.9	4.8	5.6	6.2	7.3	8.0	8.1	9.0	0.6

Penetration of fungicide into plant tissue — The results of these experiments are given in Table 4. Again WN 12, Vapam and Trapex appeared to be the most effective materials, while D-D and carbon disulphide were moderately effective. The remaining four fungicides showed little capacity to penetrate root tissue. Of the three best chemicals, WN 12, Vapam and Trapex, WN 12 and Trapex appeared to be distinctly superior to Vapam.

Activity in the soil — The results of the experiments with soil-tubes showed that all the nine chemicals tested were effective below the surface when drenched on soil (Table 5). WN 12 was easily the most outstanding, while Trapex and Vapam were next in effectiveness, and these three materials were superior to D-D. The remaining chemicals were less effective than D-D, and formalin and Fumazone especially penetrated soil poorly. However, Dowfume which showed very low vapour phase activity performed moderately well.

U. deusta appeared to be the most vulnerable pathogen in soil assays, and *P. hypolateritia* the most resistant (Table 6).

An interesting observation in these experiments was the presence of the soil fungus, *Trichoderma viride,* on the agar discs in which the test fungus had been killed (Fig. 3).

The viability of *F. noxius* could not be assayed accurately in these tests, as this fungus was rapidly smothered by fast-growing soil fungi.

TABLE 6 — *Susceptibility of four fungal pathogens of tea to nine soil fumigants in soil-tube tests.*

Mean No. of agar discs out of 90 showing viable fungus after treatment

Ustulina deusta	*Rosellinia arcuata*	*Fomes lignosus*	*Poria hypolateritia*
52.7	56.0	60.7	63.0

Significant difference ($P < 0.05$) = 1.2

Greenhouse experiment — It will be seen that of the seven soil fumigants tested in this experiment against *P. hypolateritia,* the most effective material was WN 12 (Table 7). It was significantly superior to all the other materials tested except Trapex. Trapex was the next best, although it was not significantly different from the standard chemical, D-D. D-D, Vapam, Nemagon and Fumazone were equally effective in pots, while Dowfume appeared to be the least effective.

TABLE 7 — *Effectiveness of seven soil fumigants against* Poria hypolateritia *in a pot experiment.*

No. of root segments out of five showing viable *P. hypolateritia* (transformed data)

Fumigants

WN 12	Trapex	D-D	Vapam	Nemagon	Fumazone	Dowfume
1.37	1.52	1.87	1.93	2.06	2.16	2.27

Significant difference ($P < 0.05$) = 0.39

TABLE 8 — *Toxicity of nine soil fumigants to five root pathogens of tea when tested by three different methods*

(Summarized from Tables 2, 4 and 5)

Method	Fungicide										Significant difference
I	WN 12	Vapam	Trapex	DD	Nemagon	Formalin	Fumazone	Carbon disulphide	Dowfume	Control	($P < 0.05$)
	20.3a	27.1	39.3	50.0	50.0	56.7	58.2	61.4	61.5	63.9	2.3
II	WN 12	Trapex	Vapam	DD	Carbon disulphide	Formalin	Nemagon	Dowfume	Fumazone	Control	
	1.19b	1.23	1.67	1.94	2.02	2.26	2.32	2.37	2.39	2.45	0.20
III	WN 12	Vapam	Trapex	DD	Nemagon	Dowfume	Carbon disulphide	Fumazone	Formalin	Control	
	1.8c	3.5	3.9	4.8	5.6	6.2	7.3	8.0	8.1	9.0	0.6

a Mean diameter of colony (mm)

b No. of root segments out of five showing viable fungus

c No. of agar discs out of nine showing viable fungus.

Discussion

A striking feature of this study has been the high correlation observed between innate volatile toxicity and fungicidal activity in the soil. The five most active compounds in the "Petri dish" tests, viz WN 12, Vapam, Trapex, D-D and Nemagon, were also the five most effective materials in the soil tests (Table 8 ; summarized data from Tables 2, 4 and 5), and the order of effectiveness was indentical in both cases. A close relationship between volatile toxicity and ability to penetrate root tissues was also evident, although the correlation was not as good as that between volatile toxicity and activity in soil.

The highly significant interaction between fungicides and fungi noted in the "Petri dish" tests indicates that some fungicides may be highly specific in their action. Formalin, for example, was moderately effective against *P. hypolateritia*, but had little or no effect against the other fungi. Carbon disulphide and Nemagon behaved likewise. A highly significant interaction between fungicides and fungi was also observed in the soil tests, and it should be mentioned that such specificity amongst fungicides had been noted in the past by several workers, including Zentmyer (1955) and Latham & Linn (1965).

The data from the pot experiment show that WN 12 and Trapex are more effective materials than D-D, and the superiority of WN 12 over D-D was further demonstrated in field experiments (Kerr 1966 ; Shanmuganathan 1967). Thus on the lines of greenhouse and field tests, the soil fungicide testing methods described in this study appear efficient, and a promising new material, viz WN 12, was detected in these studies, although it was later surperseded by the more active methyl bromide (Shanmuganathan and Redlich 1965).

In the chemical control of tea root diseases, it is necessary to kill the fungus embedded in the host tissue remaining in the soil after the diseased plants have been uprooted and destroyed. A potential fumigant should, therefore, possess high volatile toxicity, the capacity to penetrate root tissue and also high fungicidal activity in the soil. The experiments described were effective in providing specific information on each of these properties. Further, the methods used were simple and need no expensive equipment. For a rapid screening test to select the most promising soil fumigant, Methods I and II may be omitted and Method III modified by substituting infected root segment for agar disc inoculum. Such an assay would select compounds that penetrated soil and plant tissue and toxic to the test fungus.

The drench assay technique used by some workers (Corden and Young 1962) to evaluate soil fungicides is ideal for the measurement of chemical movement in soil water, but is not suitable for measurement of fumigant action of volatile materials. It is possible that treatment with excess water following application of fungicide might aid penetration, but it was not tried in this study because such treatment is not possible from a practical standpoint. It is likely that in the soil-tube tests penetration would have been better had the tubes been open at the bottom so that the air displaced by the fumigant could escape readily through the open end.

One of the criticisms of assays using agar disc inoculum is the necessity to use sterilized soil. However, in this study, except for *F. noxius*, all the test fungi were sufficiently fast growing so that their viability could be assayed accurately in non-sterile soil. A further criticism of the assay techniques described here could be the inability to distinguish between fungistatic and fungicidal effects. To overcome this difficulty, all treated inocula were incubated for 7 to 10 days to allow maximum opportunity for the test fungi to outgrow any possible fungistatic effect.

In the soil-tube tests, *P. hypolateritia* was more resistant to the soil fumigants tested than were *U. deusta*, *R. arcuata* and *F. lignosus*, and this seems paradoxical because in the "Petri dish" tests, it was the most susceptible fungus. This conflicting observation is inexplicable.

Acknowledgements

The authors wish to thank Mr P. Kanapathipillai for the statistical analyses and Mr D. J. M. Hettiaratchi for the photographs.

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