Studies on the White Root-Rot of Tea Bush. II.*

On the relation between pathogenicities and different conditions of inoculation, as well as on fungal growth in deficient oxygen.

By

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The writers[1] (1953) reported on the relation between pathogenicities of 3 cultural strains of causal fungus and climatic seasons. In present work, the writers have made examinations on the relation between pathogenicities and different conditions of inoculation, and on the fungal growth in condition of deficient oxygen, using the same cultural strains of causal fungus as in previous work.[1]

The writers wish to express their sincere thanks to Prefectural Laboratory of Tea Industry of Kyoto for affording them with tea seedlings used in the following experiments.

I. Difference in the pathogenicities of *Rosellinia* sp. under some conditions of inoculation.

The method of inoculation was the same as in the previous work, with the addition of disease index 1.5 for death of portion of a plant.

(1) Difference in the pathogenicity of the fungus as influenced by soil sterilization and the season of inoculation.

Experiments were made using two sorts of soils, one was untreated, and the other was sterilized for 2 hours in KOCH'S sterilizer before inoculation. There were three experiments between the period from April to November 1953. The results are given in Table 1 and Fig. 1.

From the results of the experiments, disease infection seemed to be more severed in sterilized soil than in unsterilized soil with the exception of cultural strain R 3 of the second inoculation test. With respect to the inoculated seasons, the severity to the disease was in the order of spring to summer, autumn, and winter. Difference in the pathogenicity between the cultural strains was slight, but in general, R 1 and R 3 were stronger than R 2.

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Table 1. Difference in the disease index as influenced by the soil treatment and the season in which inoculations were made.

Seasons of inoculation	Treatment: Cultural strains / Days after inoculation	Sterilized soil				Unsterilized soil			
		R1	R2	R3	Control	R1	R2	R3	Control
1st inoculation test (April 25–June 14, 1953)	24	5	5	0	0	0	2	2	0
	30	86	50	33	0	36	11	25	0
	40	97	80	86	0	75	44	58	0
	57	100	100	100	0	100	47	86	0
2nd inoculation test (June 25–Aug. 12, 1953)	16	13	5	5	0	0	0	0	0
	22	88	22	33	0	0	0	50	0
	31	88	25	17	0	8	0	80	0
	48	91	50	75	0	50	25	100	0
3rd inoculation test (Sept. 19–Nov. 13, 1953)	14	0	0	0	0	0	0	11	0
	23	36	0	8	0	13	0	13	0
	31	55	0	33	0	13	0	13	0
	40	61	0	41	0	13	0	16	0
	47	72	0	41	0	16	8	16	0
	55	77	0	41	0	16	13	16	0

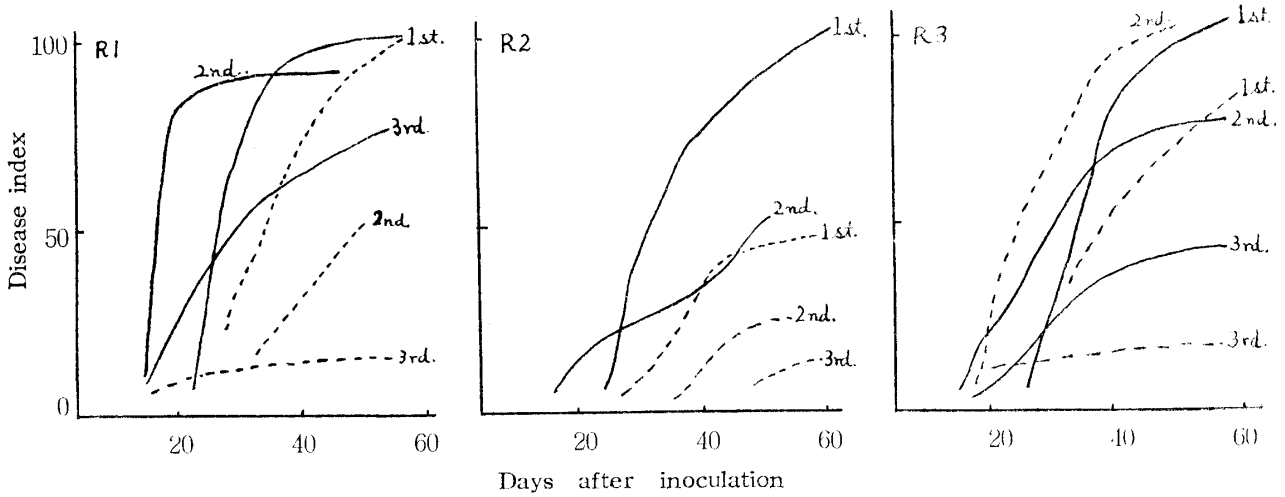

Fig. 1 The results of inoculation test in sterilized and unsterilized soils at different seasons.

Sterilized soil : ——— Unsterilized soil : - - - - - -

1st inoculation test : April 18–June 11, 1953
2nd inoculation test : June 25–Aug. 12, 1953
3rd inoculation test : Sept. 19–Nov. 13, 1953

(2) Difference in the pathogenicity of the fungus as influenced by mechanical structure of the soil, the soil moisture and the season of inoculation.

In these experiments two kinds of soils, namely clayish loam and sandy loam, were used. The clayish loam was from a paddy field of Saikyo Univ. and the sandy loam was prepared from it by mixing a quarter in its volume with sand. The soil were inoculated, and the water contents of the soils in unglazed pots were regulated by the amount of water applied the pots were set on PETRI dishes and held under a glass house. To facilitate rooting of the tea seedlings transplanted into the pots, they were given sufficient water for 4 to 5 days. When the test plants were established in the pots, they were divided to two groups of wet and dry treatments. In the wet treatment the plants were given liberal application of water so as to keep the soil at near saturation by setting the pots in the PETRI dish partially filled with water; whereas for the dry treatment the plants were irrigated with a limited amount of water enough to maintain a minimum level of vitality shown by the seedlings of the control group (uninoculated soil). As a matter of course, the amount of water used for irrigation of wet and dry groups varied with the season in which the inoculations were made.

The present experiment covered periods of seasons between October of 1952 to November of 1953.

The results obtained are given in Table 2 and Fig. 2.

Results of table 2, show the season of inoculation influenced the disease severity in the order of spring, autumn and winter, this was the same as the results described in section (1).

Between sandy loam and clayish loam, the disease seemed to be heavier on the latter than the former. Although there were some discrepancies, in general, the disease severity depends on the water content of the soil was recognized to be heavier on dry plots than on wet ones. Reactions of cultural strains were observed to be same as those obtained in section (1).

Disease index was determined by visual observation in which case disease symptoms were classified in to five grades as healthy, slightly attacked, wilting, partially dead and dead.

Days taken for the appearance of first recognizable symptom after inoculation ranging from 10 to 50 depending upon environmental conditions.

In comparison of disease severity on present work there lie many difficulties. As a standard of comparison days take to reach disease index of 50 after inoculation was thought, but it was unsuitable because the disease index in some cases did not

Table 2. Difference in the disease index as influenced by the mechanical structure of the soil and the soil moisture.

Seasons of inoculation	Soil	Sandy loam						Clayish loam					
	Cultural strains	R1		R2		R3		R1		R2		R3	
	Water / Days after inoculation	W	D	W	D	W	D	W	D	W	D	W	D
1st inoculation test. Oct. 13–Dec. 25, 1952	20	0	0	0	0	0	0	0	8	0	0	0	4
	24	0	8	0	0	0	0	0	17	8	0	0	13
	33	0	21	0	8	0	0	8	25	8	8	13	13
	39	0	75	0	25	0	29	17	50	33	25	13	33
	58	13	92	0	29	0	38	58	63	54	46	13	46
	75	17	92	0	42	0	38	67	67	63	63	13	54
2nd inoculation test. April 18–May 28, 1953	19	0	20	0	4	4	8	4	20	16	8	4	4
	24	20	50	0	25	54	37	50	83	70	62	62	66
	33	83	100	29	91	100	100	91	100	95	100	100	83
	40	87	100	41	95	100	100	91	100	100	100	100	87
3rd inoculation test. Sept. 19–Nov. 27, 1953	14	0	0	0	12	0	8	0	0	0	0	12	4
	20	0	0	0	20	29	37	12	25	0	0	37	25
	23	0	8	8	25	45	66	20	33	0	0	66	62
	34	16	41	20	54	91	95	41	87	0	20	100	83
	40	16	54	37	54	100	100	58	87	0	33	100	91
	58	20	70	54	58	100	100	79	95	12	45	100	100
	69	20	87	62	62	100	100	91	100	16	50	100	100

W : Wet soil D : Dry soil

Water supplied : Oct. 13–Dec. 25, 1952 { (W) 4.5 Liters in 73 days. 3 : (D) 1.6 ; ; 1 }

Apr. 18–May 28, 1953 { (W) 4.3 ; 32 days. 1.5 : (D) 2.8 ; ; 1 }

Sept. 19–Nov. 27, 1953 { (W) 5.0 ; 60 days 2 : (D) 2.5 ; ; 1 }

Disease index of the control : 0

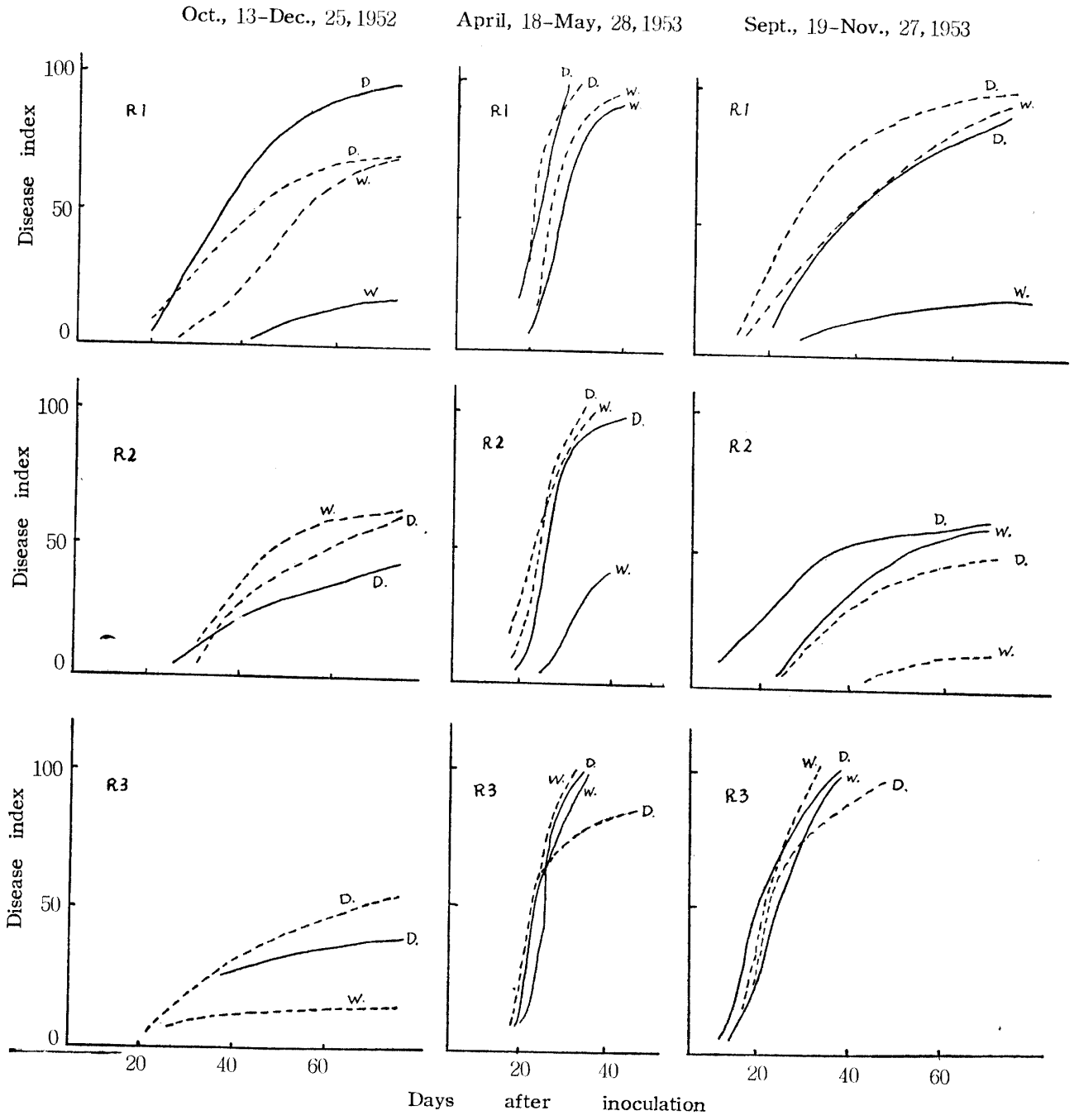

Fig. 2. Difference in the disease index as influenced by the mechanical structure of the soil, the soil moisture and the season of inoculation.

Sandy loam : ————————

Clayish loam : - - - - - - - - -

D : Dryness plot

W : Wetness plot

reach 50.

Therefore, the writers have compared the disease severity using the following method. Namely, from the disease index curves as shown in fig. 1 and 2 were calculated the angles passing through the point at 15 days. In this case the maximum disease severity would be a 90 degree. The results expressed in angles are given in Table 3.

Table 3. Comparison of disease severity by angles of average lines of disease index to latitude.

Seasons of inoculation	Oct., 13–Dec., 25, 1952									April, 18–May, 28, 1953								
Soil	Sandy loam			Clayish loam			Average by season			Sandy loam			Clayish loam			Average by season		
Water in soil	W	D	av.	W	D	av.	W	D	av.	W	D	av.	W	D	av.	W	D	av.
R1	9	47	28	35	39	37	22	43	32	69	77	73	69	77	73	69	77	73
R2	0	29	14	34	30	32	17	29	23	43	71	57	76	74	75	59	72	65
R3	0	24	12	11	31	21	5	27	16	73	72	72	74	67	70	73	69	71
Average	3	33	18	26	33	30	14	33	23	61	73	67	73	72	72	67	72	69

Seasons of inoculation	Sept., 19–Nov., 27, 1953									1952–1953								
Soil	Sandy loam			Clayish loam			Average by season			Sandy loam			Clayish loam			Average by season		
Water in soil	W	D	av.	W	D	av.	W	D	av.	W	D	av.	W	D	av.	W	D	av.
R1	10	46	28	42	50	46	26	48	37	29	56	42	48	55	51	38	55	46
R3	31	41	36	9	36	22	20	38	29	24	47	35	39	46	42	31	46	38
R3	60	65	62	67	59	63	63	62	62	44	53	48	50	52	51	47	52	39
Average	33	50	42	39	48	43	36	49	42	32	52	41	45	51	51	38	51	44

Seasons of inoculation	April, 18–June, 14, 1953			June, 25–Aug., 12, 1953			Sept., 19–Nov., 13, 1953			1953		
Treatment of soil	s.	uns.	av.	s.	uns.	av.	s.	uns.	av.	s.	uns.	av.
R1	64	58	61	65	39	52	56	18	37	61	38	46
R2	57	33	45	44	19	31	0	9	4	33	20	29
R3	57	50	53	57	65	61	39	25	32	51	46	45
Average	59	47	53	55	41	48	31	17	24	48	34	40

W.—Wetness plot, D.—Dryness plot, s.—Sterilized, uns.—Unsterilized, av.—Average.

When comparing pathogenicity of fungi, the experimental soil must be sterilized before inoculation, however, it is possible that the microbes in natural soil display synergism or antagonism with one another. NAGATA and co-worker[11](1953) reported that soil microbes inhibit growth of *Rosellinia necatrix*. In present experiments, there seemed to be an antagonistic action of some organism to *Rosellinia* sp., but this could be a result of physical or chemical change of the soil by thermotreatment.

On the reasons for heavier disease severity in spring and summer inoculation than in winter were discussed in the previous paper[1].

According to JOHNSON and HARTMAN[10] (1919), black-rot of tobacco (*Thielavia basicola*) severe disease at lower and higher levels of soil moisture, was shown but only slight at medium level. BRAUN[2](1937) described that the soil with its physical, colloid-chemical, and chemical properties, defines the conditions of existence both for the pathogens living it and hence, indirectly for the physiological disease resistance of the host plants. Also HAWKER[9] (1950) mentioned that the growth of soil inhabiting fungi is greatly influenced by moisture content of the soil.

In present experiments the optimum conditions of soil moisture for the development of this disease is not yet clear, but it appeared there was more infection in the dry plot. There seems to be a complex host-parasite relation under certain soil moisture condition as reported by CHRISTENSEN[4] (1926).

In general R1 and R3 seemed more virulent than R2. Different strains reacted differently to various chemical and physical treatments. Namely, R1 showed positive reactions on media containing tyrosine 0.1 % as well as on gallic acid 0.5 %, R3 showed some positive reactions, while R2 showed only negative reaction on the same media. As reported in previous paper[1], the optimum pH for cellulose decomposition by R2 tended toward more acid than R1 and R3. The optimum pH for cellulose decomposition coincides with that for best hyphal growth in soil, and this fact may be the reason what R2 was not as active as R1 or R3 in the soil used for the present experiment that showed a pH of about 6.2

II. The relation between oxygen supply and the hyphal growth of *Rosellinia* sp.

According to PORODKO[13](1904), BROWN[3](1922), DENNEY[5](1933), and GOLDLING[7][8] (1937, 1940) such fungi as *Penicillium*, *Botrytis*, *Fusarium*, *Alternaria*, *Neurospora* etc. are not affected by a wide range of changes in the oxygen concentration of the soil. SCHEFFER[14](1937) found the rate of decay of wood through the action of such fungi as *Polystictus versicolor* increased by increased in the oxygen supply. Many soil

fungi causing plant diseases are able to maintain activity even under a condition of little oxygen. Heavier infection by *Rosellinia* sp. generally develops in saturated or very wet and sticky soil conditions. NAGATA and co-worker[12](1952) reported that *Rosellinia necatrix* from tea bush did not survive the treatment in closed PETRI dish devoid of oxygen for 24 hours.

TERUI[15] (1954) also reported that the mycelia of white root-rot fungus isolated from apple tree lost their vitality in condition of no oxygen after 48 hours. WATANABE[16] (1954) reported that the pseudosclerotia of white root-rot fungus were able to withstand dry and anaerobic conditions. It is presumed that the mycelia of present fungus is able to maintain life and power of growth if certain amount of oxygen is supplied.

The writers made following experiments on the relation between oxygen and hyphal growth of *Rosellinia* sp. in culture. KOVACZ-ZORKOCZY[6] (1925) described that the amount of oxygen absorbed by pyrogallol is in direct proportion to the amount of pyrogallol, and is not affected by its concentration nor by the kinds or concentration of alkali used. One gram molecule of pyrogallol absorbs 2.5 atoms of oxygen.

The various required amounts of oxygen in the receptacle were calculated from the amount of pyrogallol that will theoretically absorb.

Method 1. On a slide glass supported upon VAN-TIEGHEN cell in PETRI dish, a disk of mycelium 2 mm. in diameter cut from peripheral parts of a 6 days old culture was transfered, 5 pieces to a slide. Growth of the mycelium was measured after 3 days of incubation at 24°C. When there was no recognizable growth pieces were transfered to potato extract plate culture and incubated for 7 days 24°C to determine whether they were alive.

Method 2. When mycelia of the fungus were transfered on potato extract plate medium poured into cover of PETRI dish, and incubated for 3 days at 24°C, definite quantities of pyrogallol were poured into each PETRI dishes to keep cultures in a state of regulated oxygen supply. The other treatments were same as that for the first method. Also, influence of pyrogallol and potassium hydroxide to the growth of the mycelia were investigated. The results obtained are given in Table 4.

From the results of Table 4, it appeared that the mycelium of the present fungus received a fatal injury by keeping for 3 days in closed PETRI dish devoid of oxygen. However, the mycelium remained alive in an atmosphere containing 1/10 oxygen of normal air, and continued growth even in 1/3 oxygen. The mycelial growth was markedly inhibited by the lowering the air humidity from the absorptive action of alkali solution. It is presumed that the fungus is able to persist growth in an oxygen

Table 4. The relation between oxygen supply and the hyphal growth and servival of *Rosellinia* sp. (Increase in mycelial diameter measured after 3 days in centimeters).

Cultural strains		R1		R2		R3	
Percent oxygen or treatment	Method	G (Cm.)	S	G (Cm.)	S	G (Cm.)	S
0.0%	1	0.0	−	0.0	−	0.0	−
	2	0.0	−	0.0	−	0.0	−
0.14~0.25%	1	±	+	±	+	±	+
2.3~3.5%	1	±	+	±	+	±	+
	2	0	+	0.05	+	0	+
4.6~11.5%	1	0.075	+	0.033	+	0.028	+
	2	0	+	0.1	+	0	+
(Control, water) 23.15%	1	0.6	+	0.5	+	0.4	+
	2	1.1	+	2.1	+	1.5	+
(1% Pyrogallol 5cc and water 15cc) 23.15%	1	0.6	+	0.5	+	0.4	+
	2	1.05	+	1.3*	+	1.1*	+
(10% KOH 5cc and water 15cc) 23.15%	1	−	+	−	+	−	+
	2	0.6	+	0.52	+	0.45	+

G—Growth. S—Survival.
+ : Growth and survive recognized.
− : No growth nor survival
* : Contaminated after 3 days.

content of a little below 4.6 %.

There were differences in the hyphal growths of strains in different conditions of experiment, namely a good growth was observed on R1 in the first method and R2 in the second method. Therefore, it seems that the strain R1 is different from strain R2 in its reaction to dryness. The use of pyrogallol did not cause the results to deviate greatly from the control, using water in the first method, although in the second method growth was slightly inhibited by contaminating fungi after 3 days. It may be concluded that pyrogallol has no marked influence on the growth of present fungus.

Summary

I. Difference in the pathogenicity of three cultural strains of *Rosellinia* sp. was investigated by various conditions of soil inoculation.

(1) The seasonal severity of the disease after inoculation appeared in the order of spring or summer, autumn and winter.

(2) By soil sterilizations, it was found that there exists an antagonistic condition between soil microbes and present fungus : the disease was less severe in unsterilized soil than when sterilized.

(3) More infection occurred in heavier soils; clayish loam over sandy loam.

(4) Generally speaking, the severity of present disease was greater in dry soil than in wet soil; in which a ratio of irrigated water was respectively 1 : ⅓~⅔.

(5) Cultural strains R1 and R3 were more pathogenic than R2. Pathogenicity is associated with the lignin decomposing power, and influenced by optimum pH concentration for hyphal growth and reaction of soil used in experiment.

II. The influence of oxygen supply for the hyphal growth or survival of *Rosellinia* sp. was examined by using alkaline pyrogallol. The mycelium of present fungus received a fatal injury by keeping for 3 days in closed PETRI dish devoid of oxygen. The mycelium survived in 1/10 oxygen content of normal air, and continued growth in ⅓ oxygen content of normal air.

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