AGAR PRODUCTION IN AGAR TREE BY ARTIFICIAL INOCULATION AND WOUNDING. II. FURTHER EVIDENCES IN FAVOUR OF AGAR FORMATION

M. A. Rahman
S. K. Khisa

Experiments to determine the role of wounding and inoculation of fungi in the formation of the aromatic base, agar, in the wood of Agar tree (*Aquilaria agallocha* Roxb.) were conducted in two Agar plantations at Lawachara near Srimangal. Effects of (i) inoculation and wounding, (ii) open wound with or without oleoresinous deposits, and (iii) time of creation of open wounds on agar formation have been studied. Fungi associated with the early stages of agar formation were isolated and identified.

Formation of agar in the otherwise healthy whitish agar wood can be initiated by the creation of open wounds on the trunk of Agar tree. There is no primary role of any specific fungus in the formation of agar as was previously believed. Following open wounds, microbes existing in the air spora infect the wounded tissues. The response of the host to wounding and invasion by the pioneer micro-organisms result in the agar formation. Time of creation of the open wounds has a special bearing on agar formation. Suggestions for further studies are provided.

INTRODUCTION

Experiments to determine the role of wounding alone and fungal infestation in the formation of the aromatic base, agar, in the wood of Agar tree (*Aquilaria agallocha* Roxb.) were started in two Agar plantations at Lawachara near Srimangal in 1977. Results of the first assessment of nine trees after two years indicated that the formation of agar did not depend on the activity of a particular fungus, as was previously believed, but is a general reaction of the host to injury and invasion by various microbes. It was, therefore, suggested that the effect of wounding on the

tree should be further studied (Rahman and Basak 1980). In the present study, the results of assessment of nine treated trees after about seven years and reassessment of the effect of wounding are presented.

MATERIALS AND METHODS

(a) **Assessment of the effect of inoculation and wounding after seven years :** Nine Agar trees, involved in this experiment, were inoculated in September, 1977 at Lawachara near Srimangal. The method of inoculation and wounding of the trees and the method of sampling from the trees were the same as mentioned by Rahman and Basak (1980), except that instead of collecting four core samples from four sides at 5 cm distances, two cores were collected at 5 and 10 cm from both above and below each of the five original inoculations or woundings. Thus from nine trees 180 increment core samples, approximately 10 cm long and 0.6 cm in diameter were collected in February, 1984, in sterilized test tubes. Immediately after collection, all the cores were examined for surface colouration (viz. white, light brown and dark brown) and extent of vertical spread. From the latter, the percentage of the core length discoloured was determined.

(b) **Assessment of the effect of open wounds in locations with or without oleoresin :** To determine the effect of inoculation and wounding Rahman and Basak (1980) collected 180 core samples from 45 places on 9 Agar trees in May, 1979, after about two years. In February, 1984 ten wounds were selected on each of the nine trees, five of which on each tree contained oleoresin when examined in 1979. Thus a total of 45 wounds in locations of oleoresin and 45 without were examined in the present study. At each of these 90 wounds three core samples were collected at 5, 10 and 15 cms either all above or all below the original wounds made in 1979. A total of 270 cores were thus collected and assessed for the presence of oleoresin as already mentioned.

(c) **Success of open wounds created in the months of May and September in the formation of agar :** Out of 180 open wounds created in May 1979, 12 wounds were selected so that neither at those locations nor in locations of their mother wounds created in September 1977, was there any oleoresin present. At each of these 12 wounds three cores were collected in February, 1984 at 5 cms above, below and right or left. Thus for each mother wound cores were assessed for oleoresin production as mentioned earlier. The result of this assessment was compared with the result obtained from the first assessment in 1979 (Rahman and Basak, 1980) to find out the relative suitability of wounds created in September, 1977 and May 1979 for the formation of agar.

(d) **Isolation of fungi :** Fungi were isolated from 11 dark brown, 11 light brown and 3 whitish cores collected in February, 1984. Isolation of fungi on Czapek Dox Agar (CDA), Malt extract agar (MA) and Russel's selective medium (RSM) were attempted two days after collection of samples. Small pieces of wood were also fixed on the inside upper lid of moist petridish with Blue tak. Observations were taken upto 15 days. After 20 days at 8°C mycelial growth were found on some of

the cores, mainly having light brown to dark brown oleoresinous deposits. Fungi were also isolated from the inner tissue of such cores.

RESULTS

(a) **Effect of inoculation and wounding on agar production after seven years :** There was no difference between inoculated closed wounds, using either fungus (a) *Phomopsis aquillariae* Punithalingam and Gibson (Herb. IMI No. 203954), or (b) *Botryodiplodea theobromae* Pat (Herb. IMI No. 213732) or (c) another unidentified fungus (FRI B–2) and uninoculated closed wounds (closed control) in relation to agar production. However, when uninoculated wounds were left open i. e. not covered by a polyethylene sheet (open control), a significantly higher percentage of cores contained agar (Table 1).

after nearly five years showed the fact that initiation of agar formation can be successfully obtained from simple open wounds. Wounding at sites without any oleoresin being originally present produced similar amount of agar compared to wounding at sites with oleoresin already present. In fact 64% of wounds around oleoresinous sites of 1979 extended further forming oleoresin deposits whereas 49% wounds around nonoleoresinous sites developed oleoresin. Of the cores taken at 5, 10, 15 cms, 49%, 22% and 22% of cores respectively from above or below oleoresinous sites developed agar. In contrast to this, 44%, 23% and 2% of cores at 5, 10 and 15 cms respectively from nonoleoresinous wounds developed agar (Table 2).

The results confirm the earlier finding (Rahman and Basak 1980) that there is a

Table 1. Production of agar in (a) inoculated closed wounds, (b) uninoculated closed wounds and (c) uninoculated open wounds, relationship between the three treatment with the core colours and the extent of oleoresin in nine agar trees.

Treatments	No. of cores.	Percentage of cores			No. of cores with oleo-resin	Percentage of cores with varying extent of oleoresin deposition			
		White	Light brown	Dark brown		Nil	Slight (1%–25%)	Moderate (26%–50%)	Profuse (51%–100%)
Inoculated	108	59	27	14	45	58	29	5	8
Closed control	36	75	11	14	10	72	25	0	3
Open control	36	45	22	33	20*	45	47	8	0

**Significant at P=0.02*

(b) **Effect of open wounds in locations with or without oleoresin :** Careful examination of the effect of open wounds in locations either with or without oleoresinous deposits

positive correlation between the open wounds and agar formation in the otherwise non-oleoresinous white agar wood. It is also seen that agar production extends

Table 2. Effect of open wounds at oleoresinous and non-oleoresinous sites on the formation and spread of agar

No. of trees	Oleoresin in May 1979 wounds	No. of wounds	Percentage of wounds with progressive formation (Feb. 1984)	Distance from 1979 wounds (cm)	No. of cores collected (Feb.–1984)	Percentage of cores showing agar formation			
						Nil	Slight	Moderate	Profuse
9	Present	45	64	5	45	51	40	7	2
				10	45	78	15	7	0
				15	45	78	20*	2	0
	Absent	45	49	5	45	56	33	4	7
				10	45	77	19	0	4
				15	45	98	2	0	0

**Significant at $P=0.02$*

beyond the wound sites. For example, 22 per cent of the cores taken at 15 cm from wounds at oleoresinous sites contained agar compared to only 2 per cent of similar cores taken at 15 cms from non-oleoresinous sites (Table 2).

(c) **Time of creation of wounds and agar formation :** Only seven out of 36 cores, collected at 5 cm from the 12 open wounds made in May 1979 which showed no oleoresin, were found to contain agar in February, 1984. The figure is low compared to 18 oleoresinous cores out of 36 similar cores, but taken from open wounds created in September 1977 on the same trees (Rahman and Basak 1980). More agar is, therefore, produced when wounds are made in September as compared to May.

(d) **Isolation of fungi :** *Fusarium, Penicillium, Curvularia* and *Pestalotia* species were commonly isolated. A *Pestalotia* sp. and a *Fusarium* sp. were most commonly isolated when pieces of wood were placed inside moist petridish. A *Cladosporium* sp. could be isolated only after incubation of the collected cores at about 8°C and was the dominant fungus. This fungus had loose mycelial growth. An *Aspergillus* sp. was only isolated from stored cores. *Trichoderma* and *Botryodiplodia* spp. were isolated only from one core. The results are summarized in Table 3.

DISCUSSION

The results of the present investigation support the findings of Rahman and Basak (1980) that agar formation in

Table 3. Summary of results of isolation of fungi from fresh and stored agar cores

Fungi isolated	Number of Cores								
	FRESH CORES							STORED CORES	
	Light brown cores	Dark brown cores	Whitish cores	Number of cores out of nine				Direct mycelial transfer	Inner tissue
				MA	CDA	RSM	Humid isolation		
1. *Curvularia* sp.	4	2	1	4	3	1	0	1	0
2. *Fusarium* sp.	4	5	1	5	3	0	6	1	1
3. *Penicillium* sp.	3	4	2	3	3	0	1	2	2
4. *Pestalotia* sp.	3	3	1	0	0	0	5	0	2
5. *Aspergillus* sp.	5	1	0	0	0	0	1	1	5
6. *B. theobromae*	0	1	0	1	0	0	0	0	0
7. *Trichoderma* sp.	2	0	0	1	0	0	1	0	0
8. *Cladosporium* sp.	2	3	0	0	0	0	0	0	1

otherwise healthy whitish agar wood can be initiated by open wounds. There is no primary role of any specific fungus, although following wounding microbes infect the exposed tissues and play some role in agar formation. This is shown by the fact that more agar is produced at around uninoculated open wounds compared to uninoculated closed wounds (Table 1).

Mechanical wounds and branch stubs are the most common infection courts in living trees. The exposed tissues are affected first by environmental factors such as moisture and air, then they may be infected by various pioneer organisms. Discolourations of wood tissue can be initiated by changes in atmospheric conditions. The invasion of organisms through such altered tissues may increase the discolouring processes already occurring naturally within the tree. Many times the wounds are severe enough to be infected and invaded, but an entire ring of wood may not be completely invaded. Instead. isolated streaks or islands of defect form (Shigo 1967, Shigo 1969). Under natural condition agar occurs in irregular patches and streaks in the stem wood of *A. agallocha* trees of over 25 years of age, increasing in quantity and quality with age. The best yields are found in trees which are 50 to 60 years old or more (Sadgopal 1959, Menon 1960). Centres of trees are exposed continuously to the atmosphere via the stubs of unhealed dead branches (Shigo and Sharon 1968).

Shigo (1969) pointed out that the chemical products formed when a tree is first wounded are often dark in colour due to their combination with oxygen. Such discoloured woods do not increase in area. Loranz (1944) from his studies on the successions of organisms following increment borer wounds of *Betula papyrifera* noted that discolourations were of a chemical nature even though organisms were consistently associated with wounds and that discolourations can proceed only for a limited distance without organisms being present.

Sadgopal and Varma (1952) through chemical studies conducted at Dehra Dun on the role of hydrolytic changes possible from any action on glucosidic bodies present in green and dry 'Sasi' (i. e. agar) wood revealed that it is not possible to develop agar oil or aroma by hydrolysis.

Anon (1948) recorded that the resinous contents of agar wood contain 48 percent alcohol soluble matter which include acetone, another unidentified ketone $C_{14}H_{20}O_2$, a sesquiterpene alcohol and some acids (including hydrocinnamic acid). The sesquiterpene alcohol possesses the characteristic odour of the wood.

Bacteria, hymenomycetes, and other fungi are all important in the processes that lead to discolouration and decay in living trees. Hymenomycetes invade only on those tissues previously invaded by bacteria or other fungi (Shigo 1965a).

Fungi such as *Trichoderma* spp. *Fusarium* spp., *Cladosporium* spp. *Curvularia* spp. and *Penicillium* spp. have been recorded as pioneer invaders in the process of wood decay by Shigo (1965b), Shigo and Sharon (1968) and Shigo (1971). They have also been isolated in the present study along with a few other species (Table 3).

From the experiment it may be inferred that under natural condition agar formation is initiated through the interactions caused by wounding followed by invasion of pioneer fungi which enter primarily through the openings created by broken branch stubs.

The occurrence of (a) agar in irregular patches and streaks and the scattered distribution of branch stubs on the trunk of agar trees, (b) the largely comparable nature of branch stub openings with those of the open wounds employed in the present experiments so far as the connection of air environment with the interior of agar trunk is concerned and (c) the practical feasibility of invasion by pioneer invading fungi through these types of openings are considered to adequately support the hypothesis that the oleoresinous deposits in agar trees are the result of a protective reaction of the type suggested by Shigo (1975) and Gibson (1977). The best yields of agar in trees of 50–60 years of age (Sadgopal 1959, Menon 1960) might also be associated with the relatively higher number of branch stubs on older tree as well as reduced host resistance. Hence, further studies should explicitly elucidate these and the optimum ages of agar trees at which agar formation can be initiated through artificial treatments may be determined.

REFERENCES

Anon. 1948. *Aquilaria agallocha* Roxb. In, The Wealth of India : Raw Materials. Vol. 1 : 89–90. The Council of Scientific and Industrial Research, India

Gibson, I. A. S. 1977. The role of fungi in the origin of olcoresin deposits (Agar) in the wood of *Aquilaria agallocha* Roxb. Bano Biggyan Patrika. 6(1) : 16–26

Loranz, R. C. 1944. Discolorations and decays resulting from increment borings in hardwood. Jour. of For. 42 : 37–43

Menon, A. K. 1960. Indian essential oils : a review. The Council of Scientific and Industrial Research, New Delhi

Rahman, M. A. and Basak, A.C. 1980. Agar production in agar tree by artificial inoculation and wounding. Bano Biggyan Patrika. 9(1 & 2) : 87–93

Sadgopal, D. 1959. Experimental studies on the development of essential oils and their components in aromatic plants. La France at sa parfums 3 : 62–71.

———and Varma, B. S. 1952. Agar oil from the wood of *Aquilaria agallocha* Roxb. The Indian Forester 78(1) : 26–33

Shigo, A. L. 1965a. Decays and discolorations in northern hardwoods in the northeastern United States : A consideration of micro-organisms and external signs. Paper presented at the meeting of IUFRO Section 41, October, 1965

———1965b. Organism interactions in decay and discoloration in Beech, Birch and Maple. U. S. Forest Service Research paper NE 43 : 22 pp

———1967. Successions of organisms in discolouration and decay of wood. Int. Rev. of For. Res. Vol. 2 : 237–299

———1969. The death and decay of trees. Natural History 78(3) : 43–47

———1971. Successions of micro-organisms and patterns of discoloration and decay after wounding in Red Oak and White Oak. Phytopathology 62(2) : 256–259

———1975. Biology of decay and wood quality. Proceedings of the Sessions on Wood Product Pathology at the Second Congress of Plant Pathology, Minneapolis, 1973. Springer - Vertlag, 1975 : 1–15

———and Sharon, E. M. 1968. Discolouration and decay in hardwoods following inoculations with hymenomycetes. Phytopathology 58(11) : 1493–1498

M. A. Rahman, Divisional Officer and S. K. Khisa, Junior Research Officer, Forest Research Institute, Chittagong, Bangladesh