CONTRIBUTIONS TO THE STUDY OF SPIKE-DISEASE OF SANDAL (*Santalum Album*, LINN).

PART XVI.—DISTRIBUTION OF ARSENIC IN SANDAL-WOOD TREATED WITH SODIUM ARSENITE.

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It was shown in previous communications (Varadaraja Iyengar and Rangaswami, *Indian Forester*, 1934, **60**—under publication) that the spread of spike in an area is considerably checked by killing out diseased plants which act as source of infection and that arsenical tree-killers are highly effective for that purpose. Arising out of this finding, it was considered necessary to know whether any of the applied arsenic passed into the scented heart-wood of the treated plants and, if so, whether the essential oil derived therefrom contained the poison to such an extent as to render it unsafe for use for therapeutic and other purposes. The present enquiry was therefore undertaken to determine the distribution of arsenic in the different parts of the treated plants.

Materials—A number of plants which had been previously treated with known quantities of sodium arsenite (4 lbs. As_2O_3, 1 lb. NaOH in 1 gallon of solution with small amount of added phenol) were pulled out some time after their death and divided into trunk, branches, main root and side roots. Each of these was again further sub-divided into bark, sap-wood and heart-wood. In the case of root-bark especially, adhering soil impurities had to be removed. The final materials were powdered after air-drying and stored. The samples thus obtained were mainly derived from spiked sandal but a few specimens from similarly treated healthy trees were also included for purposes of comparative study.

Methods of Analysis.—The following method was adopted to estimate arsenic :—Known quantities (usually 10 g.) of material were treated with a mixture of nitric and sulphuric acids (10 : 1) and after the complete cessation of frothing, digested until no trace of carbonaceous matter was left behind. After removing excess of nitric acid, by dilution with water and concentrating repeatedly, arsenic, which was in solution, was determined by different methods, depending on the quantities present. When the quantity was of the order of 50 parts per million and above, it was precipitated as magnesium arsenate (Treadwell and Hall, *Analytical Chemistry*, 1919, Vol. 2). When the quantities were less, it was reduced by zinc and dilute sulphuric acid to arsine, and estimated by the extent of colour produced in standard test papers soaked in mercuric chloride. Both micro and macro methods were adopted for this purpose (Scott, *Standard Methods of Chemical Analysis*, 1927, Vol. I).

The reliability of these methods was checked by adding known quantities of arsenic to different plant materials and verifying the estimates thus obtained. By this means, it was found that the added arsenic could be estimated correct to under 1 per cent. The entire digestion process was carried out in a kjeldahl flask, the acid mixture being added at regular intervals. Addition of hydrogen peroxide (5 per cent.) at periodical intervals was helpful in hastening the decomposition of organic matter. It was found necessary to remove the last traces of nitric acid as also excess of sulphuric acid to obtain accurate estimates of arsenic by colorimetric methods. Incineration of the plant material, with a mixture of equal quantities of sodium and potassium nitrates, was tried without success. Oxidation with perchloric acid was avoided for fear of loss of arsenic as the chloride.

Quantities of solution applied and the efficiency of tree-killer in different girth classes.—For this purpose, the trunk was girdled near the base, to a height of half the girth, the bark removed in this portion and the arsenical solution applied with a brush. The results of the treatments conducted in one of the experimental areas is given in Table I.

TABLE I.

Size in inches ..	2—5	6—9	10—13	14—16	17—20	21—24	25—30
Quantity of solution in ozs. ..	1/16—1/12	1/12—1/10	1/10—1/8	1/8—1/6	1/5	1/4—1/3	1/2
Percentage of death in 5 weeks ..	80	50—60	60—75	50—60	60	80	70

The tree-killer is fairly effective, though the potency can be and has subsequently been improved considerably. The quantity of solution applied bears roughly a direct relation to the size of the plants—the higher girth classes requiring more of the solution.

Distribution of arsenic in the various parts of the treated plants.—The following figures were obtained for the transverse distribution of arsenic in a few representative cases (Table II) :—

TABLE II.

Condition of the plant	Distance from the treated portion in ft.	Arsenic (As_2O_3) as parts per million		
		Bark	Sap-wood	Heart-wood
Spiked	10·0	600	250	60
"	2·0	1,720	1,120	210
"	0·0 (Treated portion)	No bark	5,960	320
Healthy	0·0 (Treated portion)	do.	8,460	290
"	2·0	3,490	790	100
"	3·0	1,610	560	80

It may be seen that the bark contains always a higher proportion of arsenic than the corresponding sap-wood, while the heart-wood contains the least amount. It appears therefore that the spread of the poison is largely through the conducting tracts of the plant.

Vertical gradient of arsenic in the arsenite treated plants.—It has already been shown that the poison travels a considerable distance from the point of application and that in the case of trees which are completely dead, arsenic may be traced even in the smaller roots (Varadaraja Iyengar, *Planters' Chronicle*, 1934, **29**, 240). In the present study further evidence is adduced to show that arsenic moves not only above the treated part along the woody tissues, but also down to the roots chiefly through the bark. The following Table (III) would illustrate the vertical distribution of arsenic in two treated plants which were selected at random and analysed.

TABLE III.

Distance (in ft.) from the point of treatment		Arsenic as As_2O_3 in parts per million			
		Bark		Sap-wood	
		I	II	I	II
Above	15 ..	120	210	70	130
	10 ..	470	520	110	220
	8 ..	680	760	230	350
	5 ..	820	1,090	360	490
	3 ..	940	1,420	420	680
	2 ..	1,080	2,150	750	960
	1 ..	2,840	3,430	1,040	1,650
Treated part, *i.e.*, zero	..	No bark	No bark	4,180	5,610
Below	6 in. ..	2,480	2,810	1,450	2,280
	12 in. ..	1,725	2,180	1,080	1,550
	1 ft. 6 in. ..	1,320	1,860	640	1,010
	3 ft. ..	840	1,270	460	790
	4 ft. ..	160	980	120	530

In the case of underground parts, big side roots were selected for study, in view of the difficulty of collecting all the roots of the plants at a given depth. This procedure was further justified by the fact that sandal is largely a surface feeder.

In the immediate vicinity of the treated portion, the tendency of arsenic movement would be more downward than upward. It is clear that the gradient recorded is not strictly proportional to the distance from the point of application. It is of interest to observe that shortly after application of the poison, the leaves turn flaccid, developing a characteristic brown colour. Moreover, the external symptoms of death in the foliage and at the growing points resemble the wilting condition consequent on insufficient water supply. It is probable that the effect of the poison is localised in the treated part, the water conduction being inhibited.

It may further be noted that thin roots (less than 2″ in thickness) contained only traces of arsenic. It has also been found that in a number of cases, arsenic had travelled to root-suckers of the treated parent plants and killed them. It would appear in these cases, that the poison had moved farther through some roots than others.

Arsenic in the scented-wood and oil.—Examination of a number of specimens of heart-wood from treated plants showed that all of them contained arsenic though in minute quantities. The results are presented in Table IV.

TABLE IV.

Specimen Number and Condition of Sandal	Arsenic as As_2O_3 in p.p.m.	Specimen Number and Condition of Sandal	Arsenic as As_2O_3 in p.p.m.
1 spiked	320	7 spiked	40
2 ,,	180	8 ,,	180
3 ,,	210	9 ,,	160
4 ,,	100	10 healthy	210
5 ,,	290	11 ,,	350
6 ,,	80	12 ,,	270

Although the quantities of arsenic passing into the heart-wood are very small, it still appeared probable that traces of the poison may pass into the oil and thus affect its quality. With a view to verifying this, samples of the wood were powdered to pass the 40-mesh sieve and the oil extracted by both the solvent and the steam distillation methods. In the former case, the method of Briggs (*Ind. and Eng. Chem.*, 1916, 8, 428) was adopted. In the latter, the oil was distilled with steam at a pressure of 25-30 lbs. for nearly 5 hours. The specimens of oil obtained by both the methods were digested with acid mixture and the arsenic in the digest estimated in the manner already described. Careful examination of a dozen samples showed, however, that not even a trace of the poison was present in any of them. The reliability of this conclusion was tested independently as follows: Experiments with known quantities of arsenic added to the oil under study showed that the same could be estimated correct to under 1 per cent. These observations show conclusively that, in spite of the presence of arsenic in the heart-wood of the treated plants, none of it passes into the oil derived from it.

It became of interest, however, to know whether arsenic comes at all during distillation with steam. To this end, the distillate after the separation of the oil was concentrated to a small volume and digested with acid mixture and arsenic estimated in the extract. It was found that a small quantity of the poison does pass over, the amount varying from 7 p.p.m. to 20 p.p.m. In a few instances, no trace of this constituent could be detected (Varadaraja Iyengar, *Half-Yearly Reports on Spike Disease of Sandal*, Pt. VII, 1933). The reason for the presence of arsenic in the distillate lies probably in the possible transfer of fine particles of the scented wood with steam. There is, however, no indication that oxides of arsenic are steam volatile. It follows therefore that all the arsenic is in the residue only. Moreover, the water is discarded in the process of collecting the oil. It may further be pointed out that the only possible manner in which the poisonous element can enter into the composition of the oil is through the formation of organic derivatives of arsenic. The analyses conclusively prove that such derivatives are not formed. Thus the quality of the oil is not impaired, in any manner.

Transmission of the poison to root-suckers.—In the course of some experiments conducted in N. Salem forest areas, it was observed that in addition to treated plants some untreated ones also showed signs of arsenic poisoning and died. A careful examination of the roots of the latter, revealed that they were suckers originating from the treated ones, showing thereby that the poison may have been transmitted to them through the connecting roots. Since this observation is of great practical interest, the roots of the treated plants, as also those of the suckers, were examined for their arsenic contents (Table V). In both cases the root specimens were derived 2-3 ft. below ground, at a distance of 5 ft. from the respective plants.

TABLE V.

Arsenic as As_2O_3 in p.p.m. in the roots of			
Treated Plants		Root-suckers	
Bark	Sap-wood	Bark	Sap-wood
480	230	180	60
610	340	240	80
560	240	170	50

DISCUSSION.

The mechanism of death in plants resulting from arsenic poisoning has not been adequately studied. When arsenic as arsenate is added in minute quantities to the soil, an increase in growth of plants is generally noticed. In larger amounts the plants die preceded by intense withering of the foliage. Among the various arsenic compounds, sodium arsenite is the most potent, acting even in concentrations of 1 : 250,000,000. It would appear that the poisonous action proceeds from the roots, in which the protoplasm is disorganised, hindering osmotic action, resulting in the final death of the roots. According to Mathieu (*Ann. Falsif.*, 1912, **5**, 78) more arsenic is detected in grapes and wine from vines sprayed with arsenicals than from untreated ones, thereby indicating the translocation of the poison to different parts. A more descriptive account on the subject is presented by Brenchley (*Inorganic Plant Poisons and Stimulants*, 1927). Recently Crafts and Kennedy have studied the conditions that determine the effectiveness of chemicals in weed control (*Plant Physiol.*, 1927, **2**, 503). In a later contribution, these authors (*ibid.*, 1930, **5**, 329) followed the course of arsenic when applied to the leaves of *Convolvulus arvensis* (Morning-glory). They have found the presence of arsenic even in the distant roots, though only in small amounts. It is well known that the effectiveness of a plant poison is dependent upon penetration and subsequent distribution in the several tissues. These factors are more important when the destruction of diseased tissues which act as source of infection is concerned. In the case of arsenic, penetration is best effected by the incorporation of acids or bases which hasten diffusion of the poison. But the distribution of the poison is influenced by the mode of application, the strength or potency of the chemical and finally, by the physiological condition of the plant.

In the present study, the plants were girdled, the bark removed to expose the woody portion, and the solution smeared on it. By this means it was hoped that the poison would reach both the foliage and the roots acting on its way. Mere girdling of the plant to expose the wood, does not result in its death, while in presence of the toxic material, the aerial parts die quickly. The manner in which this is brought about will be considered here. An examination of Table III will show that both the sap-wood and bark of either stem or root, contain large quantities of arsenic, while the heart-wood contains far less amount (Table II). Moreover, a gradient in arsenic content is visible though it is not strictly proportional to the distance from the region of treatment. Two possibilities may be considered in explaining the mode of transfer of arsenic. Primarily, the poison applied at the exposed woody part moves along the transpiration stream and traverses down the bark with the organic nutrients from the leaves.

The accumulation near the 'ring' may then be due to discontinuity of the bark. Alternately, it is possible that above the girdle, arsenic is transferred to the bark as it moves along the transpiration stream. This may explain the progressively decreasing concentration of arsenic in the distant aerial parts. Arsenic has also been detected in the leaves, but no excessive accumulation has been noticed. Of the two theories regarding the movement of arsenic, the latter one seems more plausible when considering that with the progress of disease, accumulation of starch takes place in stem and leaves inhibiting the movement of food materials. Experiments with cut shoots in dilute solutions of sodium arsenite have failed to show the presence of arsenic in the bark. Below the girdle, however, arsenic movement has not been very rapid. A great accumulation has been noticed near the girdled part, while the distant roots do not have much arsenic in them. The defective translocation, so characteristic of spike, is perhaps responsible for this. The conditions that determine the penetration and distribution of arsenic below the 'ring' are still obscure. A critical analysis of the various factors will form the theme of a later contribution.

It is significant to find that in all the cases examined the concentration of arsenic is greatest in the bark, less in the sap-wood and least in the heart-wood (Tables III and IV). These observations show that the poison travels or is present in considerable quantities only in the conducting tracts of the growing plant. The extremely low value in the scented wood is due to the fact that it is a dead tissue and as such, transfer of the poison therein is rather poor. On the other hand, a lateral diffusion to the bark tissue is possible in view of the nearness to the woody portion and the large amount of alkali in the solution applied.

It is well known that root-suckers from spiked sandal are generally diseased, thus acting as source of infection. The penetration of the poison even to the suckers shows its high efficiency in killing out diseased material. The amount of poison in them is, however, far less than that of the parent plants (Table V).

Although the poison penetrates rapidly into the bark tissue, it is low in amount in the heart-wood. The complete absence of arsenic in the oil derived from the treated trees, establishes convincingly that the adoption of arsenical tree-killers does not in any way affect the use of the oil for therapeutic and other purposes.

SUMMARY AND CONCLUSIONS.

1. In arsenical treated spiked sandal plants, large quantities of the poison are found to be present in the bark and sap-wood.

2. Movement of the poison has been noticed even in the roots at some distance from the point of application.

3. The greatest concentration of arsenic is found near the girdled region.

4. Root-suckers of treated plants are also often killed out, due to transfer of the poison to their roots.

5. In spite of the presence of minute quantities of arsenic in the heart-wood of treated plants, the oil derived from them does not contain any of the poison, irrespective of the method of extraction. The application of arsenicals for control of spike-disease is thus free from objections.

6. The mechanism of death due to arsenic application appears to be due to effective penetration and thorough distribution of the same immediately above, in and below the girdled portion, killing the tissues and thus inhibiting nutrient supply.

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