FIELD STUDIES IN THE SPIKE-DISEASE OF SANDAL (*Santalum Album* LINN).

I. Observations on the Natural Dissemination of Spike.

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A CLOSE understanding of the manner in which the natural dissemination of an infectious disease occurs, is often helpful in elucidating the nature of the vector and in devising practical methods for an effective control of the disease under field conditions. In the case of sandal spike, a considerable amount of valuable information has accumulated not only on the mode of its incidence and spread under field conditions, but also on the probable nature of the vector, yet undetermined.

It is recognised that spike-disease exhibits two types of spread, (1) the sporadic outbreaks affecting isolated patches of sandal far removed from any apparent sources of infection, and (2) the secondary attacks responsible for the radial spread of disease around the centre of primary infection. A number of primary attacks involving long distance flights of disease have been recorded in the official reports of the Forest Officers, the most remarkable instances of them (Table I) are cited by Lushington (1918). Rama Rao

TABLE I.

	Site of Primary attack	Distance from the nearest source of infection miles	Year of attack
1	Thalli	12	1915
2	Tholwabetta	14	1915
3	Mahadeswaranagudi ..	14	1915
4	Kollimalais	40	1915
5	Javadis	100	1916

(1918) draws attention to the rapid spread of disease over localities far away from its original location and adds that as found by Mr. Muthanna in 1902-03, "the disease was restricted to isolated tracts here and there in Hunsur Taluk and parts of the Mysore and Heggadadevanakote taluks adjoining Hunsur".

This discontinuity in the natural spread of spike has been so intriguing that many have doubted the infectious character of the disease. Hole (1918) contended "that the disease is not really infectious", on account of (1) "the frequent occurrence of isolated cases of spike several miles from any infected trees", and (2) "the fact that trees quite close to spiked individuals not infrequently remain free of spike for long periods". Lushington was inclined to believe that the disease may be endemic in origin. Rama Rao (1918) has remarked that Coleman's grafting experiments have only demonstrated "that the disease is communicable from tree to tree when in close organic contact, but they have not yet reached the stage of explaining the spread of disease in Nature from one area to another with an intervening unaffected space of many miles between" and is led "to be sceptical as to the contagious or infectious nature of the disease and to believe that, after all, it may be endemic and spontaneous".

To explain this long distance "skip overs" of spike, many theories have been advanced. McCarthy (1918) believes that the infection is "carried from spiked *Zyzyphus* and other species that have already spread to other areas", but Lushington (1918) pertinently remarks that "even if we prove that sandal can be infected by other species, we still have to prove how the disease is caused in those species". Coleman (1917) discussing certain possibilities of such isolated outbreaks of spike, refers to the probable carriage of infection through seeds. Although several experiments in seed testing have been carried out by a number of Forest Officers, the question has not been studied critically. Seeds from the spiked portion of a partially spiked plant, dropping off prematurely, do not germinate while seeds from the healthy portion, ripen to full maturity and on germination, give rise to healthy plants. Lushington (1918) has recorded a few germination tests; but the cause of the death of the plants raised has not been noted and no sign of spike was observed. Germination tests of Hart and Rangaswami (1926) revealed all the seedlings which succeeded in establishing themselves continued healthy. No explanation has, however, been offered for the large number of seedlings which died during their experiments.

Recently, Bewly and Corbett (1930) have obtained 6·04 per cent. of infected seedlings—a result which could not be so far secured by standard methods. Experimenting on the seed transmission of the "Y" potato virus in *Solanum nigrum*, Smith (1933) records successful transmissions with ripe but undried seeds which, on desiccation, appeared to lose their infective power, indicating that the virus was "either adsorbed to the outside of the seed coat and could not be removed by washing or was enclosed within the seed coat and was inactivated by desiccation". Duggar (1930) discussing the problem of seed transmission of tobacco mosaic attributes the failure to transmit the disease to the adsorption and inactivation of the virus by the reserve proteins of the seed. The bean mosaic is known to be transmitted through seeds and a number of other instances of seed transmissions, like the mosaics of *Dolichos biflorus*, *Pisum sativum* and Cucumber, have recently been reported.

Sandal spike appears to be seed borne.—Our germination tests revealed (1) that generally seeds derived from the spiked portion of a partially spiked plant did not give rise to any seedlings, while (2) those from the healthy portion gave rise to seedlings which have since remained perfectly healthy. That these plants neither carry nor mask the infection was proved by grafting and defoliation tests. This is a result to be expected in view of the fact that spike virus has been definitely shown to be localised (Sreenivasaya, 1930) in certain portions of the plant and that spike is not a systemic disease in the strict sense of the term. Seeds if affected in the very early stages of maturity dry up and do not germinate.

In one instance, however, the seeds were obtained from an artificially grafted plant whose fruit-bearing branch manifested symptoms of disease when the fruits were in advanced stage of maturity. These seeds on germination gave rise to two spiked plants which died two months after. This is the first instance of seed transmission of spike, a possibility long suspected by various workers in the field. Seeds of established maturity should be collected on a more extensive scale from spiked plants in forests and the virus-bearing character of the seeds determined by germination tests. As Ray Nelson (1932) points out with regard to the seed transmission of bean mosaic, the percentage of infection among such sandal seeds may turn out to be very small and irregular. In all such cases even a single positive result should be considered significant and justify the enforcement of appropriate precautionary measures.

The primary attacks can now to a certain degree be explained by the possible carriage of such seeds through birds or animals, and seeds ordinarily not capable of germination may germinate easily by their passage through the alimentary canal, where the virus-bearing seeds might even acquire greater virulence. The possibility of the carriage of seeds through the agency of birds and animals has been recognised by Coleman, Lushington and others. Hearsey (1918) records that "the flying fox carries the fruit and eats and deposits the seed at long distances". Excreta of monkeys and wild cats have been found to contain sound sandal seeds possessing a high germinating power. Seeds of *Zyzyphus œnoplia* are disseminated through animals and birds in a similar manner and since the Zyzyphus spike is found to be seed borne by us, its spread may be explained by the carriage of seeds in this manner.

The possibility of infection through pollen has been invoked by Hart and Rangaswami (1926) because of the fact that spike is spreading in an East-North-Eastern direction corresponding to the general direction of the prevailing winds during the south-west monsoon. They further observe that sandal plants under natural conditions have never become spiked before flowering but no experimental transmisions have so far been achieved through pollen, and recently a number of young sandal plants have got spiked under natural conditions before flowering. It is therefore highly improbable that the disease is carried through this agency.

Insect Vector of Spike.—McCarthy (1918) observed that insects may act as carriers and from the nature of the spread of infection thought the insects had only a short radius activity. His opinion was based on the fact that the spread of disease had been temporarily checked by clearing a belt of sandal and *Zyzyphus œnoplia.* Lushington looked upon the insects as the direct cause of spike since he found spike-like symptoms on several species of plants closely associated with insects; further Hearsey (1918) produced spike-like growth in *Argyreia cuneata* with the aid of the red spider, which has been found to be "distributed by wind, coolies' clothes and probably by other agencies among which Mr. Hearsey mentions herds of cattle". Coleman (1917) held that insects are not the originators but merely the carriers of the virus.

A considerable amount of indirect evidence has accumulated strongly pointing to the existence of an active insect vector responsible not only for the long distance disseminations of disease but also for the gradual spread of infection in a given area. The comparative sluggishness exhibited by insects need not necessarily be opposed to its long distance flights with the aid of winds, birds and other animals. Chapman (1926) draws attention to the important rôle played by the horizontal and vertical air movements, in the dispersal of insect fauna and fungi. Spores of fungi have been found at an altitude of 11,000 ft. and are reported to be often carried from Kansas to Wisconsin. Pollen grains have been found at a height of 16,000 ft., swarms of mosquitoes at 3,000 ft. and grasshoppers at 2,000 ft. (Felt, 1925); young larvæ had been carried across Cape Cod Bay, a distance of 19 to 30 miles. Quayle (1916) found that the young of the black scale, *Saisethia oleæ*, is distributed by the wind from one block of trees to another.

Elton (1927) refers to a few remarkable instances of long distance carriages of aphids and hoverflies with a strong gale of wind for a distance over hundred miles. He also refers to animals and other agencies constituting the means of transport for a great many species of other forms of animal life. Hautefuille (1926) records instances of lac insects being "accidentally transported to long distances, isolated colonies of *coccus lacca* having been found here and there as far as La-pho". Hautefuille and Mahdihassan have both observed that certain flies frequent the colonies of *coccus* during the time of swarming, entangle themselves with the larvæ and help their dissemination. The lac insect is also carried by strong winds and through the agency of birds.

Dover (1931) refers to the construction of an "elaborate insect trap for studying the effects of wind directions on the dispersal of insects in sandal areas" but at the moment we have no access to the results which would be most valuable.

The next observation of importance in the natural spread of spike is that the disease mostly attacks trees which are situated in denuded areas and enjoy exposed positions. Further, these attacks have occurred in close proximity to agricultural fields and colonies. Lushington (1918) noticed that the first outbreaks of spike often occur in "exposed positions". In the case of the Javadis, spike appears to have developed first at the South-West corner which is the most exposed position of the forest. "In this connection he discovered to the west of the area some waste fields which were cultivated some years back." Rama Rao (1918) observes that "when the disease puts in its first appearance in undulating country, the trees first affected are those growing on exposed knolls or ridges with hard and strong soil", which has been largely confirmed by our own observations. In Coorg two primary attacks in artificially raised sandal plantations, Meenkolli and Bambookadu, appeared at the very edge of the plantation. Mitchell and Rangaswami (1931) record a number of "sites of initial attack" occurring in close proximity to cultivation and the outbreaks have occurred almost invariably at the edge of cultivation which also offers an "exposed position". Sreenivasaya (1931) cites a number of instances of primary attack in Mysore and North Salem which have occurred at the "edge of cultivation".

The frequent association of agricultural fields and abandoned sites of cultivation with the primary outbreaks of spike is an observation, the significance of which has been discussed at some length by Sreenivasaya and Rangaswami (1931). The association of agricultural operations and settlement of colonies in a forest area results in a denudation of the area, establishment of new crops, new weeds, new diseases and new insects which may be disseminated by herds of cattle grazing in the forests close by. Sreenivasaya and Sreenivasa Rao (1931) state that the weeds invading the area suffer from mosaics and leaf curls, significantly absent from healthy areas. Several of them were found associated with aphids and Jassids.

To determine if the leaf curls and mosaics of weeds had any connection with spike, grafting tests on sandal were carried out, employing the diseased leaf tissue. There was no transmission of any disease symptoms on the operated sandal plant. The transmission of one of these diseases including "spikes" of other species through the agency of an insect vector, should, however, be suggested as a possibility. But the parallel spread of these diseases with the spread of spike, however, is highly suggestive.

The most important among the weeds considered to influence the incidence and spread of spike is Lantana. McCarthy (1918) thought that Lantana influenced if at all the spread of spike in an extremely indirect manner. He cites instances of spike outbreaks in areas in Kollegal and North Coimbatore, where not a single lantana plant existed. He also refers to perfectly healthy areas in which sandal is associated with heavy growths of lantana. Lushington (1918) considered lantana as a "harbourage for insects harmful to sandal and urged a systematic search for the vectors of infection". Tireman's (1918) experiments on the effect of lantana removal on the incidence of spike led to a significant diminution in the number of attacked trees. Hart and Rangaswami (1926) confirmed these results but did not offer any explanation. Sreenivasaya and Rangaswami (1931), as a result of their ecological studies, showed that lantana is fifteen times as abundant in spike areas as in a corresponding healthy area, and discuss the rôle of lantana in predisposing sandal areas to disease. (1) The aggressive spread of lantana suppresses the growth of the comparatively slow-growing and deep-rooted species having considerable host value to sandal; (2) Owing to its shallow root-system and its being one of the earliest to shed leaves with the approach of drought, lantana does not provide the sandal parasitic on it, with a continuous and unfailing supply of nutrition; (3) Lantana is easily susceptible to fires, especially during hot weather, and thus contributes towards a greater frequency of fires in the area leading to a gradual annihilation of all the species useful to sandal. A few areas in Jowlagiri, in fact, represent practically a pure patch of lantana with a few *Acacias* and sandal plants struggling in their midst; (4) Insects are found to increase in lantana-infested areas in both variety and abundance; (5) Bird-life which would tend to restrict an abundance of insect fauna, is scarce in lantana-infested areas, as the areas become unattractive to birds due (*a*) to scarcity of food and (*b*) to danger from fires; and (6) The toxicity imparted to the soil by lantana growth affects the growth of other plants in the areas.

Continual weeding out of lantana brings about a remarkable change in the floristic composition of a given area. The ecological change brought about by the keeping down of lantana in Cairn No. 53 at Jowlagiri during the last 10 years, may be cited in this connection.

TABLE II.

	Natural order	Number of plants	
		Lantana-free	Lantana-infested
1	Acanthaceæ	61	2
2	Apocynaceæ	14	0
3	Bixaceæ	99	11
4	Capparidaceæ	9	3
5	Combretaceæ	8	0
6	Convolvulaceæ	108	0
7	Diascoreaceæ	31	2
8	Ebenaceæ	26	3
9	Euphorbiaceæ	82	3
10	Leguminoceæ	99	17
11	Liliaceæ	92	7
12	Lythraceæ	6	0
13	Melastomaceæ	108	15
14	Meliaceæ	9	0
15	Moraceæ	11	2
16	Oleaceæ	809	111
17	Rhamnaceæ	140	30
18	Rubiaceæ	139	23
19	Rutaceæ	162	3
20	Sapindaceæ	26	0
21	Tiliaceæ	27	0
22	Verbinaceæ	14	1
23	Lantana	0	2303
	TOTAL ..	2,080	2,536

A comparative floristic survey of the two areas (Table II), lantana-free and lantana-infested, has revealed that out of the 21 natural orders occurring in the areas, 7 are exclusive to the lantana-free area; about 4 or 5 of them are practically absent from the lantana-infested area; with regard to the common species occurring in both the plots, there is a decided preponderance of them in the lantana-free plot which one can easily observe to be well stocked not only with sandal but also with host trees.

Insect Scars and their Significance.—Extensive observations have been made on the incidence of scars (first observed by Rangaswami) inflicted by insects and other fauna associated with sandal areas. These scars were found not only on sandal but also on other species like lantana, *Scutia indica, Zyzyphus œnoplia, Acacia pennata, Pterolobium indicum, Odena* sp. (?). It was found that *Erythroxylon monogynum* and *Memicylon edule* were sparingly attacked.

Fresh scars in large numbers commence to appear about the first or second week of April, usually after the first heavy showers, and continue to be inflicted on the young shoots of sandal until the end of the following July. Further, scars are inflicted mostly during the early hours, before dawn; this is a significant observation helpful in determining the vector of spike.

It has been suggested that the number of scars in a given area may be taken a rough measure of the relative abundance and activity of insect fauna obtaining in spiked and healthy areas. It should, then, be possible to correlate disease incidence with scars.

TABLE III.

Healthy areas		Spike areas	
Mahadeswarangudi, N. Salem	218	Sameri grafting plot, Aiyur, N. Salem	2,085
Devarabetta, N. Salem	180	Thalli R.F., N. Salem	574
Meenkolli, Coorg	171	Chinnanahalli, Coorg	593
Banavara, Coorg	172	Tittimatti, Coorg (Fresh outbreak)	315
Doddamalathe, Coorg	102		

It will be seen from Table III that per 100 ft. length of shoot examined, the healthy areas, in general, have a lower number of scars than the corresponding spike areas. Lantana removal lowers the incidence of scars on sandal and the same holds true in the case of areas well stocked with host plants. This does not necessarily mean that there is a corresponding decrease in insect population; it may be due to the fact that the insects are provided with other hosts of equal, if not superior, food value. These observations have a practical bearing in the control of spike and should be confirmed by more extended studies.

Platform Experiments.—In November 1931, one of the sandal plants, two years old, artificially regenerated at Jowlagiri got spiked, and this attack was followed by others in the same area. During the same period it was observed that the disease was spreading in an epidemic form in almost all the observation and experimental areas in North Salem. There was thus a fine opportunity to obtain more information regarding the vector. Experiments were accordingly carried out in Sameri grafting plot where the infliction scars and the incidence of spike were progressing in a particularly virulent form.

Platforms (distributed at random) were erected in the area, to the height of the lantana bush. Batches of 16 to 18 pot-cultured sandal plants, raised from seeds collected from perfectly healthy sandal plantations, were periodically sent to the area for being kept on the platforms for a definite period, after which they were replaced by another batch of new plants. These plants which were exposed to infection on platforms for a period, were brought back to Bangalore (50 miles away) and kept in the nursery of the Institute free from any natural infection. These experiments were continued till July 1933 and the results are given in Table IV.

TABLE IV.

	Period of exposure to natural infection	Number of plants spiked
1	April–June 1931	2
2	April–June 1932	2
3	October–December 1932	2
4	April–June 1933	1

It is clear from the table that April-June appears to be the principal season during which the natural infection proceeds in forests, while a second season of infection during October-December is also indicated by the results. April-June, as has been shown previously, is also the season during which fresh scars are inflicted on sandal.

Among the batches of plants exposed to infection during other periods of the year, there has been no incidence of spike in spite of the defoliation carried out to force out the masking symptoms if any. None of the plants kept on the ground within the lantana bush got spiked.

This result establishes (1) that agencies responsible for natural infection operate above ground and are active and virulent during the period April-June, (2) that an intensive entomological survey during this period during the early hours before dawn, should prove fruitful in determining the vector, and (3) that the vector is possibly screened off by the bushy growth of lantana, thus confirming the observation made by previous investigators, that a sandal plant under cover has, so far, not been found spiked.

Experiments on the Caging of Sandal Plants.—The screening effect of the lantana bush suggested that an artificial caging of the sandal plants might effectively ward off the vectors and prevent spiking. 115 healthy plants were accordingly selected for the purpose in the 6-acre regeneration plot at Jowlagiri and caged in March and April 1932 with muslin reinforced with a bamboo structure. The plants had been previously defoliated and shown to be free from masked symptoms of disease. After caging they were all fumigated with Chloropicrin. The large number of plants interspersed among the caged plants served as controls. One of the caged plants was found to be spiked on 26–6–1932, about 2 months after caging, which is obviously due to an infection before caging; while 22 among the control uncaged plants had got spiked. By July 1934, *i.e.*, after two years and four months, it was found that none of the caged plants had got spiked while 13·4 per cent. of the control plants succumbed to the disease thereby showing the effectiveness of caging. Similar experiments in the Nognoor area confirm the above findings, 9·5 per cent. of the uncaged and none among the caged plants having got spiked.

Grafting under Sylvicultural Conditions on Big Sandal Trees and Its Significance.—Grafting of big sandal plants in various girth classes was carried out in the Kenilworth Castle area, mainly with the object of determining the incubation period in various girth classes and to find out if there was any difference in the resistance offered by sandal plants at their different ages of growth. The selected trees were grafted in May 1932, each plant receiving a dose of 4 patches and 4 leaves. The plants succumbed to the infection with unexpected rapidity, the average incubation period (the period elapsing between infection and manifestation of disease) being 92 days. About 80-100 milligrams of fresh tissue was found effective in spiking even big trees, if the tissue were placed at the vulnerable point and grafted during the susceptible season. It has been found that April, May and June are usually the months during which the sandal plant appears to be most susceptible to infection. This is corroborated by the experiments on the monthly graftings of pot-cultured sandal plants in the Institute nursery. The dosage of infective material for successful spiking is so small that its carriage by insects is not improbable.

Summary.

1. The discontinuity in the natural spread of the disease and the consequent occurrence of isolated outbreaks of spike in areas far removed from all apparent sources of infection is explained on the basis of the possible

dispersal of (1) the virus-bearing seeds and (2) the viruliferous vectors, whose long distance transport is facilitated by wind and other agencies.

2. The possibility of spike virus being transmitted through seed has been experimentally indicated and explained in the light of the recent work of Bewly and Corbett, Duggar, Ray Nelson, Smith and others. A more extended study of this question is suggested.

3. The frequent association of agricultural fields or abandoned sites of cultivation with the primary outbreaks of spike, has been established in almost every case that is examined. The denuding effect of agricultural colonies and their operations on the forests close by and the exposed positions which sandal plants occupy in consequence, are suggested to predispose the sandal plants to disease.

4. The parallel spread of mosaics and leaf-curls among the associated flora (weeds) with the spread of spike is highly suggestive and indicates that the environment is favourable for the multiplication and spread of viruliferous vectors.

5. The rôle of lantana in relation to spike disease is discussed.

6. Attention is drawn to the occurrence of insect scars during the months of April, May and June, and its usefulness, as a measure of insect activity in a given area, indicated.

7. The season during which the vector is most active and virulent, has been determined by the platform experiments extending over two years. The results show that the period April-June is the principal season during which the natural infection vigorously proceeds in forests, while a second season of infection during October-December is also indicated by the results.

8. Experiments on the caging of sandal plants growing under sylvicultural conditions in a highly infected area, show that caging will effectively screen off the vectors. The fact that none of the caged plant has succumbed to the disease during a period of now over two years, shows that root transmission plays a very minor rôle in the spread of spike under natural conditions.

9. Experiments on the grafting of big sandal trees have shown that even small doses of infective tissue (80 to 100 milligrams), if introduced at the vulnerable point during the susceptible season, are adequate to spike even big trees of sandal. The quantity of the material is so small that its carriage by a swarm of insects is not improbable.

10. April-June appears to be the most critical period in the life of the sandal plant. During this period insect fauna becomes visibly active in sandal forests and there is a heavy infliction of scars on the tender shoots of sandal. The sandal plant is most susceptible to infection during this

period and if a practical means could be found to protect them during these months, there is every chance of the plants remaining "safe" for the rest of the year.

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