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Germination and penetration studies on coffee rust (*Hemileia vastatrix* B. & Br.)

By R. W. RAYNER
*Coffee Research Station, Ruiru, Kenya**

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SUMMARY

Spore dispersal, germination, penetration and incubation-period studies on coffee rust (*Hemileia vastatrix* B. & Br.) in Kenya, East Africa, are described.

Evidence is produced that air-borne spores may be trapped effectively on the upper surfaces of leaves, thence to be liberated and transported to the undersurfaces of other leaves by rain splash.

Germination requires liquid water and was observed to occur in 2·6–4·7 hr. (medians) at 23° C., the minimum being 1 hr. Appressoria were formed in 6·5–8·5 hr. (medians) with a 5·3 hr. minimum. Germination is inhibited by light and in the field by the rapid evaporation of water droplets on the lower leaf surface which occurs during daylight. Light inhibits the growth of germ tubes that are less than 30 μ long and reduces rate of growth if they are longer; appressoria may continue to form.

In the field, appressorium formation and infection can occur between 10 p.m. and 8 a.m. If coffee trees are wet at dusk or rain falls before midnight, infection is probable and it is proposed that the number of occasions this occurs be used to forecast the severity of the annual rust maximum.

The median incubation period throughout the year varied from 4 to 7 weeks, increasing with low temperatures and dry conditions. A multiple regression of mean maximum and minimum temperatures on incubation period gave fair agreement between observed and computed values for Ceylon and Mysore. Susceptibility and incubation period were strongly affected by coffee variety and rust biotype, but not by age of leaf or crop size.

INTRODUCTION

In the Kenya Highlands annual attacks by *Hemileia vastatrix* on *arabica* coffee vary considerably in intensity. However, to control the disease by fungicides it is essential to apply a protectant spray before it is known whether a serious attack will develop. The writer has observed that unseasonable rains in January to February promote rust outbreaks later in the year. This relationship has recently been used to forecast outbreaks and issue warnings when sprays should be applied.

To improve the technique of forecasting the studies on germination and penetration described below have been carried out at the Coffee Research Station, Ruiru (altitude 5400 ft.).

Marshall Ward (1882) reported that at 24° C. *H. vastatrix* spores germinated in

* Present address: Commonwealth Mycological Institute, Kew, Surrey.

12–24 hr. and appressoria were formed in about 48 hr. At laboratory temperatures at Peradeniya, Ceylon a yellow area appeared on the infected leaf after about 14 days and sporing commenced 2–4 days later. Germination occurred only when spores were in contact with water, and penetration was via stomata, which are present only on the lower surfaces of the leaves. Using 'Vaselined' slides he demonstrated the spores to be airborne and considered that they alighted mainly on the upper surfaces of the leaves, thence to be washed round to the lower surface by rain.

Mayne (1933) in Mysore, southern India, confirmed many of Ward's findings. He found that light strongly inhibited germination, an observation also made by Bürk (1867).

EXPERIMENTAL

Spore dispersal

Ward (1882) showed that spores were liberated into the air and blown for considerable distances. To produce infection they must reach the stomata-bearing lower surface of the leaves. Using the spore-cloud method for depositing dry spores under a bell-jar (Morgan, 1960), the writer found none were deposited on the lower leaf surface. Spores deposited on the upper surface were dislodged by blowing only when a strong current of air was directed through a narrow jet placed about 0·3 mm. from a spore. If droplets of water were placed on the leaf the majority of spores floated to the surface. Thus, air-borne spores falling on leaves would be temporarily held on the upper surfaces from which they could be removed by rain.

The wetting of the undersurface of coffee leaves during rain storms was studied in the field. Some wetting resulted from raindrops landing directly on the undersurfaces when the leaves were disturbed by turbulent winds. Rain also flowed round the margins of the leaves on to the undersurface. However, most of the wetting was by raindrops rebounding from the top surfaces of lower leaves. Such splashes would carry with them spores deposited on these surfaces. That a drop of rainwater sometimes passes through a lesion, picks up spores and flows on across the leaf surface is suggested by the occasional occurrence of lines of small, young lesions running out from an old one.

Germination

Time required

With *in vitro* tests using hanging drops in Van Tieghem cells nil or very little germination was observed in diffused daylight, but it was abundant in the dark. In the light, when it did occur, the process took longer and the germ tubes were very stunted. In the dark at 19–20° C. it started after 2–3 hr., at 25° C. it took 2·3 to 3·5 hr., and at 30° C. it was slower and much reduced or nil.

Germination studies by earlier workers have been confined to glass substrata. To observe germination and appressorium formation on leaves and at various time intervals circular areas were marked on them and inoculated by stroking with a damp spore-bearing paint brush. After light spraying with water the inoculated leaves were placed in Petri dishes lined with damp filter-paper and were supported just clear of the paper by pinning their bases and tips to pillars of Plasticine. The inoculated areas were removed at intervals, one from each of the three leaves used, and examined for germination by drying them in a current of air and making a cellulose acetate cast as

described by Bennett & Furmidge (1956), but staining with Congo Red, which was found more satisfactory than Safranin.

In a preliminary test 98·8 % germination was found after 3 hr. The results of three further tests are shown in Fig. 1. Plotting probits suggested a somewhat skew distribution. Hence the results were summarized as median germination times which were 2·6, 3·7 and 4·7 hr. for the three tests. 5 % germination was estimated as occurring approximately at 1·0, 1·8 and 1·0 hr. and 95 % at 5·2, 6·2 and 9·8 hr. In one test, some germination was observable after only 1 hr. Hence, the process must start almost immediately the spores are wet and at 22° C. may be considered to be complete for practical purposes in about 7–10 hr.

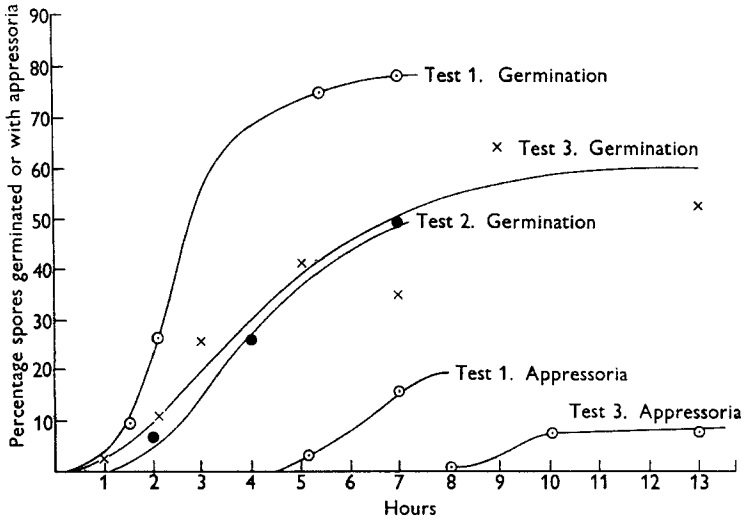

Fig. 1. Progress of germination and appressorium formation with time for *Hemileia vastatrix* spores on the undersurfaces of wet coffee leaves in the dark at 23° C.

Appressorium formation occurred in all tests, but was infrequent in the second. The earliest observed was at 5·3 hr. and probably did not occur in less than 4½ hr. A median could only be estimated in one test and was about 8½ hr. with 5 and 95 % points at roughly 7½ and 10 hr., respectively. The maximum percentage of germinating spores producing appressoria was only 19·6 %.

The necessity for free water

The effect of high humidities on spore germination at constant temperature (25° C.) was investigated. Cover glasses for Van Tieghem cells, glass slides and leaves were sown with spores using Morgan's (1960) method. Slides and leaves were supported above moist filter-paper as before. In replicates the filter-paper was moistened with concentrated solutions of lead nitrate and sodium sulphite in contact with the solid phase in order to produce 98 and 95 % R.H., respectively. Wherever salt-free water was used, it condensed on the spore-sown surfaces and germination resulted. On the leaves appressoria were produced by up to 87·5 % of the germinated spores.

Germination was found on one of the leaves only at 95% R.H. but it was only incipient and abnormal. None occurred at 98% with either leaf or glass surfaces.

During other investigations on living leaves when liquid water disappeared from the surfaces inhibition of germination always resulted. It is concluded that high humidity alone is not sufficient to stimulate germination.

The effects of light

Studies by earlier workers on the effects of light have been confined to glass substrates. To determine whether germination could occur on inoculated leaves with full out-door illumination a few were sprayed and placed in Petri dishes and supported above damp filter-paper. Moisture rapidly disappeared from the lower surfaces of the leaves, even though the weather was mainly dull. No germination occurred. Sprayed leaves on branches enclosed in polythene envelopes containing a pool of water behaved in the same way, even when cool air was bubbled through a sintered plate in the pool.

Inoculated 1 cm.^{2} leaf disks were floated, lower surface uppermost, in Petri dishes nearly full of water in an incubator with a Perspex door in front of a laboratory window. After allowing a period for equilibrium to be be reached, they were sprayed. Even then, some loss of water was observed after 2 hr. No germination was detected.

Leaves in the field were inoculated and sprayed during drizzling rain in daylight. The lower surfaces quickly dried and were resprayed repeatedly. Even during heavy rain, drying out occurred within 45 min. No germination was found.

Germination in daylight in the field is thus not only adversely affected by the light itself but also by evaporation of water droplets. Satisfactory conditions for germination will presumably only normally occur at night.

To test whether the growth of germ tubes that had started in the dark would be inhibited by light, inoculated leaf disks were floated in Petri dishes as described above and kept in the dark. Germination was estimated on a sample and the length of the longest germ tube on twenty germinated spores measured. Half the remaining disks were then exposed to light.

In one trial the average germination after 3 hr. in the dark was 7·7%. After a further $1\frac{3}{4}$ hr. it was 6·0% in the light and 18·0% in the dark. Thus at 3 hr. it was incomplete and continued in the dark, but was inhibited in the light. The average length of germ tubes was 78 μ for those continuously in the dark and 94 μ for those exposed to light for part of the time. Thus growth had continued in the light.

In another trial, after 4 hr. dark, germination continued during a further $2\frac{1}{2}$ hr. in the dark, but not in the light. Average germ tube length after 4 hr. was 72 μ and after $6\frac{1}{2}$ hr. 146 and 86 μ respectively. Thus further growth occurred in the light but less than in the dark. By examining the probit frequency distribution curves for germ tube lengths, it was deduced that germ tubes which were less than 30 μ long on exposure to light were completely inhibited from further growth. Above this length there was decreasing inhibition with increasing length and none above 110 μ.

The results indicate that germination commencing before dawn will not continue, though germ tubes may go on growing if they have reached 30 μ in length.

Germination and appressorium formation in the field

In a series of trials, leaves on a large potted plant in the open were inoculated using a damp paint brush to pick up spores and distribute them over the lower surface. At a set time or times they were lightly sprayed with water and together with the subtending branch enclosed in a polythene envelope. Spraying was done at various times from 8 a.m. to 10 p.m. and examinations, using the cellulose acetate method, were made at 10 p.m. the same day or 8 a.m., 6 p.m., 8 p.m. or 10 p.m. the next day.

Nil or very low germination was recorded by dusk for spores wetted in the morning. Considerable germination occurred between 6 and 10 p.m., but it was not usually completed in this time. Only in one case out of four was germination by 10 p.m. increased by the spores having been wet during the day. Appressorium formation was detected the following morning at 8 a.m. when wet conditions commenced at 6 p.m. and in three trials when they commenced at 10 p.m.

With one doubtful exception there was no increase of germination from continuing moist conditions through the next day, but in one trial a statistically significant increase in percentage of appressoria was found. By the evening most of the germ tubes had disintegrated. Thus if rain falls by 10 p.m. appressorium formation may be expected by the next morning and may continue during that day if wet conditions are maintained. From the laboratory observations, it is likely that a period of at least 3–4 hr. of wet conditions before dawn is necessary before appressorium formation can commence, and rain would have to fall not later than 2 a.m. If it falls later, but at least 2 hr. before dawn, and wet conditions continue after dawn, infection may perhaps occur. The possibility needs further investigation.

The period required for infection

Leaves on bushes in the field were each inoculated by transferring spores to five small water-droplets placed near the mid-rib and between the main lateral veins. Pairs of old, medium-aged, and young leaves were inoculated on each branch, which was then lightly sprayed with water and enclosed in a polythene sleeve. Different branches were inoculated at 1 p.m., 6.30 p.m. and 10 p.m. The sleeves were removed from half the branches at 8 a.m. the following day and from the remainder at 8 a.m. the day after. The leaves then rapidly dried off. During enclosure moist conditions

Table 1. *The percentage of inoculated points developing rust lesions on leaves maintained moist for various periods*

Moist period		Percentage developing rust lesions	
Commenced	Terminated 8 a.m.		
Monday 1 p.m.	Tuesday	20	10·9
	Wednesday	3	
Monday 6 p.m.	Tuesday	5	11·8
	Wednesday	18	
Monday 10 p.m.	Tuesday	7	7·0
	Wednesday	7	
Total	Tuesday	11·0	—
	Wednesday	8·7	—

were maintained by periodic respraying. The inoculated areas were recorded for rust lesions 7½ weeks later and the percentages showing them are given in Table 1, which shows that maintenance of moist conditions from 10 p.m. to 8 a.m. was sufficient to produce infection and that no increase in percentage infection resulted after a further 24 hr. of moist conditions. The percentage of areas infected on old, medium-aged and young leaves was 12·7, 9·5 and 9·1 %, respectively. There was thus no evidence for young leaves being more susceptible.

The incubation period

Observations on the seasonal variations in the incubation period were made on three trees cropping well and three poorly. Inoculations were made as described above at intervals of 14–28 days on two young and two old leaves per branch.

On some occasions only pale-yellow, non-sporing areas were produced. It is noteworthy that this was most noticeable under drought conditions when the incubation

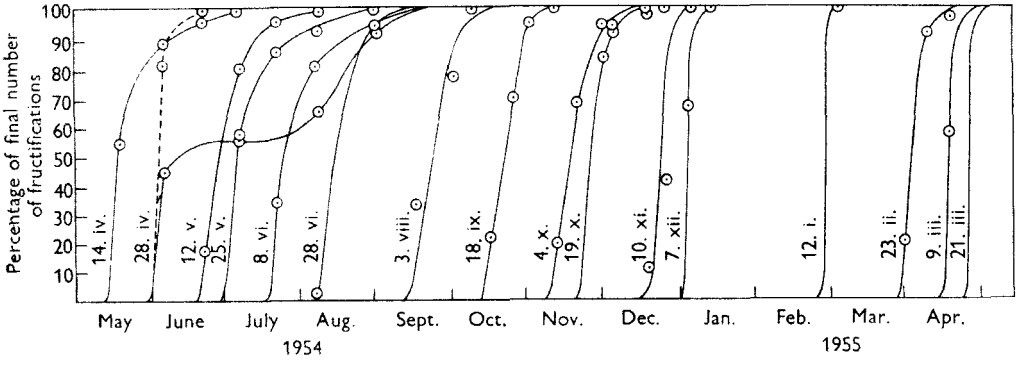

Fig. 2. The progress with time of sporing fructification development expressed as a percentage of the total number finally developing for inoculations carried out at various times during the year. The respective date of inoculation is noted on each curve.

period was also very long. Lesions resulting from inoculations on a given date did not all appear simultaneously. Therefore the number of inoculated areas with sporing lesions was expressed as a percentage of those finally developing. These were plotted against time and curves fitted by eye (Fig. 2), from which the time required for 5, 50 and 95 % to sporulate was estimated. The difference between the 5 and 50 % points varied with inoculation date from 1 to 9 days and that between the 50 and 95 % points from 1 to 32 days. The time to reach maximum spore-producing activity, roughly estimated, varied from 6 to 13 weeks and sporing was largely finished in 7–18 weeks in the absence of leaf shedding.

The effect of date of observation on the 5 and 50 % points is shown in Fig. 3. Apart from the rather anomalous very long incubation period for the inoculation of 12 January it was longest during May to September, the cooler months.

It was often possible to relate rust outbreaks in the field during these observations to specific dates of infection. Thus the rust maximum in early April could only have been induced by a rain shower on 20 February, this being the only rain that month. This incubation period, 5½ weeks, was the same found by inoculation. The only

heavy showers previous to a strong development of young spots on 13 September were on 28 and 29 July, giving $6\frac{1}{2}$ weeks incubation. The first heavy storm of the 'Short Rains' on 8 November was followed by a strong development of spots on 12 January, a $4\frac{1}{4}$ week period. The only marked fall of rain the month before the next outbreak on 9 March was on 4–6 February, $4\frac{1}{2}$ weeks previously. The following outbreak at the end of April was preceded by the beginning of the 'Long Rains' in the third week of March, suggesting a 5-week incubation. Most of the periods deduced agree well with expectation from the inoculations.

The percentages of successful inoculations showed no statistically significant effects of age of leaf or size of crop on the trees.

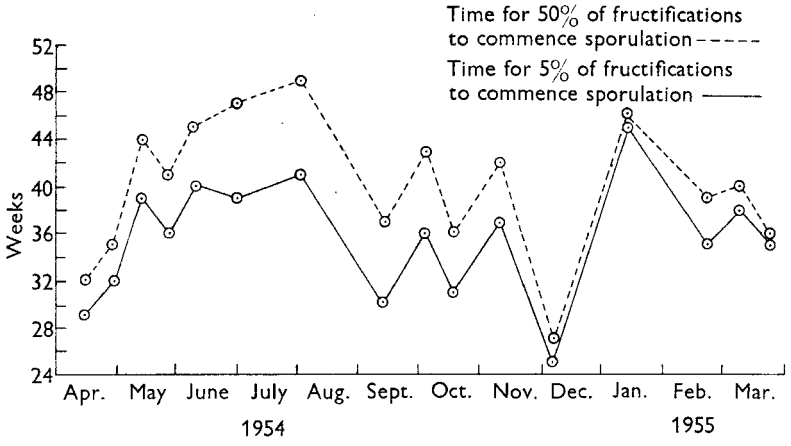

Fig. 3. The variation of incubation period with time of the year.

The relationship between temperature and incubation period was investigated. As seasonal variations of temperature in Kenya are not adequately characterized by average temperatures the mean maxima (X_1) and minima (X_2) during the incubation period were used to calculate a multiple regression equation, $Y = 90{\cdot}61 - 0{\cdot}408X_1 - 0{\cdot}440X_2$, where Y was the estimated incubation period (50% point).

The agreement between observed and calculated periods was best from May to August. The main departure was that of the inoculation of 12 January, when the period was much longer than estimated, probably due to drought. No spore production from that of 9 February occurred, although yellow spots resulted. The other main departures were in the early 'Long Rains' and the late 'Short Rains', the periods being shorter than expected. Both were periods of heavy rains and thus may represent the opposite to the drought effect.

Incubation periods for points near the upper and lower altitude ranges of coffee planting in Kenya have been estimated from temperature data. The averages were around 33 days for Makuyu (4800 ft.) and 37 and 38 for Upper Kiambu (5700 ft.). Although the difference is not large, it may be critical in determining the number of generations possible in a given length of rainy season and hence the rate of disease build-up. In addition in cold weather in Upper Kiambu the period increases to 42 days.

The incubation periods found for Kenya are very considerably longer than those

reported from elsewhere. Ward (1882) reported 15 days for old leaves and 10–11 for young ones to produce yellow areas, sporing requiring 2–4 days more. His observations were apparently made in a laboratory at about 78° F. Mayne (1933) at Ballehonnur, Mysore, South India, also working under laboratory conditions, found 15–24-day incubation periods. Estimates calculated from the equation on p. 503 for the temperatures which applied showed that these were probably largely responsible for the shorter periods found.

In testing for varietal susceptibility and for the determination of rust races much information on incubation periods in the laboratory in the open and in a greenhouse was accumulated. The range observed was 19–63 days with race I (*sensu* D'Oliveira (1955–57)) and 26–48 days with race II. The longest periods with race I were found with the coffee variety K7, which is immune to race II.

Considerable differences between varieties were observed, up to 17 days with race II and 28 days with race I. On the whole those giving shorter periods with one race also did so with the other. However, their relative position differed. Thus Amphillo gave rather shorter periods than Harar with race I, but considerably longer ones with race II. With both varieties and with both races they were shorter than with SL34.

The high field susceptibility of Harar may in part be related to the very short incubation period shown by both rust races on this host. Another factor is the greater number of lesions which it shows from the same degree of inoculation. The reverse occurs with K7, which is susceptible to race I, but at Ruiru it was very difficult to obtain successful inoculation and the incubation period was very protracted. During the warmer seasons and in the greenhouse, inoculation was more frequently successful and the incubation period shorter. In the field at Ruiru (5400 ft.) and at the Scott Laboratories, Nairobi (5900 ft.), true K7 plants are not attacked. At lower altitudes, however, a fair infection may develop on K7, but it is never so strongly attacked in such localities as other varieties such as SL. 34 and SL. 28, which are susceptible to both races I and II.

DISCUSSION

In the recent reviews by Wellman (1957) and Razafindramamba (1958) 24 hr. is stated to be necessary for germination and Bürk's (1887) finding of a 2–2½ hr. minimum has been overlooked. The present observations are in accord with those of Bürk and the whole process of infection has been shown to be complete in 10 hr. and may possibly take less time.

In the literature young leaves are frequently said to be more easily infected than old, a statement probably mainly originating with Bürk (1887). However, he apparently carried out no inoculations to compare susceptibility and based his view on considerations as to which leaves remained wet long enough for infection to occur. The present investigations do not support his conclusions. Further, in numerous determinations of race or varietal susceptibility, young leaves were found difficult to infect, possibly largely because water tends to run off them very easily. No difficulty was found in infecting mature leaves of any age. With natural infection young lesions have been observed on leaves of all ages except those still of juvenile (glossy) appearance. This appearance is lost at between 5 and 23 (average 12·2) weeks of age (Rayner, 1951).

As the incubation period averages 5 weeks it is evident that leaves are rarely infected until fully expanded.

From the present investigations it is clear that germinations can normally occur only at night. Infection will also occur at night or possibly during the following day if the leaves remain wet. Its amount over a period of days will be related to the number of nights during which water droplets are present on the undersurfaces of the leaves. In producing these, rain is more important than dew, according to the writer's observations in the East Rift of Kenya, since the latter has been rarely observed to form on the lower leaf surfaces and only then usually shortly before dawn. The number of nights on which leaves are wet at nightfall, or in which rain falls before 10 p.m., may be taken as a lower limit of the number of occasions on which infection is likely to occur. Once wet at dusk, the leaves remain so until the following morning. An upper limit may perhaps be fixed by the number of nights rain falls before 2 a.m. For practical purposes in forecasting rust outbreaks the difference between these upper and lower limits will be small and probably of little importance. Further investigation could fix the upper limit more precisely and determine whether other factors, such as the physiological state of the plant and seasonal variation in other meteorological factors have any significant effect on the time required for infection.

A more detailed account of these investigations is deposited at the Coffee Research Station, Ruiru, Kenya and at the Commonwealth Mycological Institute, Kew, England.

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REFERENCES

BENNETT, S. H. & FURMIDGE, C. G. L. (1956). Spray application problems: XXIV. The study of plant surfaces and spray deposits by means of transparent impressions. *Annu. Rep. Long Ashton Res. Sta. for 1955*, p. 121.

BÜRK, W. (1887). Over de Koffiebladziekte en de middelen om haar te bestrijden. *Meded. PlTuin, Batavia* **4**, 6.

D'OLIVEIRA, B. (1955–57). As ferrugens do Cafeeiro. *Rev. do Café Português.* **1** (4), 5; **2** (5), 5; **2** (6), 5; **2** (7), 9; **2** (8), 5; **4** (16), 5.

MAYNE, W. W. (1933). Annual Report of the Coffee Scientific Officer, 1931–1932. *Bull. Mysore Coffee Exp. Sta.* no. 7.

MORGAN, N. G. (1960). A simple suction apparatus for the uniform deposition of dry fungal spores on detached leaves. *Annu. Rep. Long Ashton Res. Sta. for 1959*, p. 126.

RAYNER, R. W. (1951). *Kenya Dept. Agric. Annu. Rep. 1949*, Vol. II, p. 49.

RAZAFINDRAMAMBA, R. (1958). Biologie de la rouille du Caféier. *Rev. Mycol., Paris*, **23**, 177.

WARD, MARSHALL H. (1882). Researches on the life history of *Hemileia vastatrix* the fungus of the coffee leaf disease. *J. Proc. Linn. Soc. Bot.* **19**, 299.

WELLMAN, F. L. (1957). *Hemileia vastatrix*—Investigaciones presentes y pasadas en la herrumbre de café y su importancia en la America Tropical. *Fedecame: Sección de Divulgación*, no. 23, 1957.