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A REPORT TO THE UNITED STATES AGENCY FOR INTERNATIONAL DEVELOPMENT ON LETHAL YELLOWING DISEASE OF COCONUT IN JAMAICA

BY

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Abstract

The United States Agency for International Development Project 532-0000 Technical Support F and N, Lethal Yellwoing (LY) Research, Under a contract with the University of Florida, aimed at study and identification of palm feeding insects as possible vectors of the LY.

Preliminary work included procurement of research equipment, conversion of existing facilities in Coconut Industry Board and the University of West Indies for the necessary research work, a general survey of homopteran insects in coconut orchards in diseased and healthy areas of the island, and the preliminary testing of Epoasca sp., Dawnaria sp., Apoganalia sp., and Eriophyes sp.

Most of the research consisted of radioactive labeling of some coconut trees, trapping and identifying the insects labeled b feeding on the coconut palms, and studying the translocation problems of the diseased palms as means of effective labeling of insects or detecting the diseased palms at early stages.

The movement of solutions throughout healthy and diseased palms was determined by injecting radioisotope 32-P into the tree, then sampling various tissues at time interval, after injections. Leaf disks were excised from leaflets and counted by a proportional gas counter. Stomatal openings were measured by the silicon impression method.

No significant radioactivity was detected in the leaves of the early stage diseased trees in the dry season when petioles or the trunk were injected with 32-P, or when whole trees with intact roots were placed in 32-P and water solution except for slight activity in the youngest fronds.

In the wet (rainy) season the translocation of 32-P in the trees were inversely proportional to the advance of the LY disease.

Injection of 32-P in the tips of the fronds in a diseased tree failed to produce radioactivity in lower leaflets or the spear leaves, and the phloem feeding homopteran, Haplaxius crudus, picked up radioactivity from the diseased trees after a longer period of time as compared to the healthy controls pointing to a blockage and delay in the phloem flow.

Secondary roots, which appeared healthy on visual examination, were excised from an LY-diseased palm and their tips placed in a 32-P solution. Uptake by these roots was four-fold less than that in roots excised from healthy palms.

Observations of stomates in diseased and healthy palms indicate that no. of open stomates on green leaflets were inversely proportional to the advancement of the disease, even at times of the day when stomates on healthy palms are open. These data suggest that xylem flow is severly limited in palms with lethal yellowing, even in early stages of visible symptom development.

Radioactive isotope 32-P was used to label 45 healthy non-bearing Jamaica Tall variety of coconut tress in Jamaica from January 15 to May 15, 1979--in Caenwood during the dry, and in Plantain Gardens during the rainy season. About 10,000 homopteran insects were trapped on and around these palms by sticky light, yellow, and cone traps. These homopterans were placed on x-ray films daily for initial screening, and after 3 days of exposure, the labeled insects were analyzed with a gas-flow counter for radioactivity.

Autoradiographs of labeled insects were evaluated by assigning a 1-10 visual index of light transmission intensity. In addition, homopterans were collected by a D-vac insect sampling machine from the undergrowth and vegetation in the vicinity of the coconut palms and were caged on radiolabeled coconut leaflets.

Among the insects fed on palms and caught in traps, 22 species, and 7 genera have been tentatively identified, and 5 specimens are unknown. Ten species and one genus were identified to have fed among those confined on radiolabeled leaflets. Total no. of labeled insects of each species collected ranged 1-26, with their radiation counts per minute between 17-235; background counts between 12 and 38.

Some species collected with higher numbers and radioactivity have not been previously tested in lethal yellowing transmission studies, or not adequately tested. These include Dawnaria sordidulum (Muir), Idioderma varia (Van Duzee), Typhlocybella minima Baker, T. maculata n.sp., Cedusa wolcotti (Muir)., Cedusa sp., Agalliopsis tropicalis (?), and several species of the genus Empoasca. We suggest that phloem feeders of above insects be extensively tested for LY transmission in Jamaica.

The coconut palm or "tree of life" produces food, drink, fuel and shelter in the form of 90 different products used in domestic economies of millions of people in the tropics (Grimwood 1975). World acreage planted to coconuts is estimated at 10,000,000. The annual world earnings from copra (oil and animal feed) were estimated by Child (1974) to be $435,000,000, of which 90% was from the Philippines, Indonesia, Malaya, Ceylon and Oceania.

Coconut plantings in Jamaica cover an estimated 62,000 acres with 89% in less than twenty-five acre, owned by 9,965 farmers. In 1977, 3,734 tons of copra were produced, down from 20,400 in 1967 (Coconut Industry Board Five-Year Development Plan, 1978-1983). The earnings were calculated to be Jamaican $1,38 million in 1977 instead of a potential Jamaican $9.75 million based on 1967 tonnage. Nearly 60% of this loss is attributable to the lethal yellowing disease (LY) of coconuts (Plate 1); the remainder is due to low rainfall (30%) and mite damage (10%).

History of the Lethal Yellowing Problem

The earliest reports of LY were in Jamaica (Fawcett 1891), Key West in 1955 (Corbett 1959) and West AFrica in 1948 (Meiffren 1948). Since then it has spread to Cuba (Romney, D. H. and H. C. Harries, Rept. on a visit to Cuba 1978, Coconut Industry Board, Jamaica), Haiti (Romney and Harries 1978), The Dominican Republic (Schieber 1970) and Florida (Fisher 1975). In Florida 375,000 palms have succumbed (Gwin 1978) including over twenty species of ornamental palms (Thomas 1978) which are important to Florida's tropically-oriented tourist industry. In Jamaica it is estimated that the susceptible

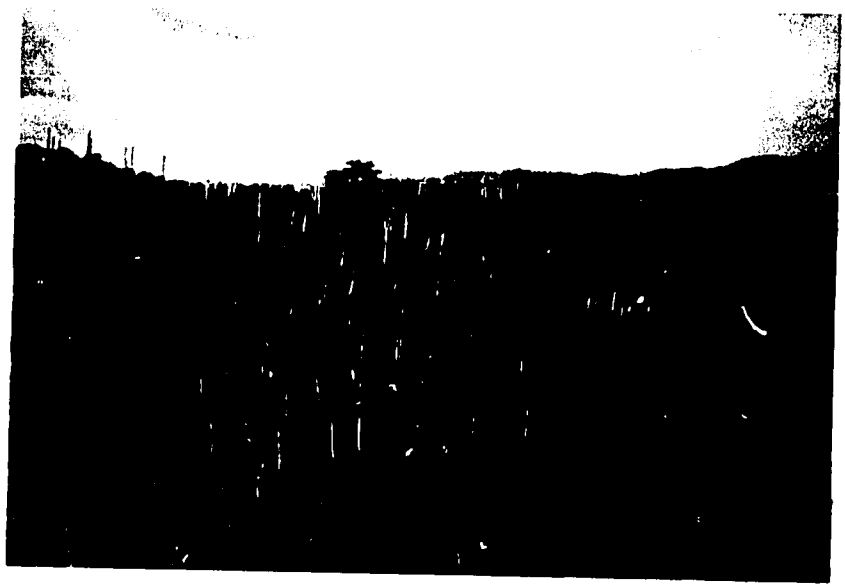

Plate 1. Coconut plantation after the lethal yellowing disease; Plantain Gardens, St. Thomas, Jamaica

varieties of "Jamaica Tall" coconut trees have been dying at the rate of 1,000 per day over the past seven years. In Ghana LY is considered to be the most serious disease of coconuts and a threat to production on the African Continent (Addison 1978).

Lethal Yellowing Disease

LY is an infectious disease of the coconut and at least twenty-three other palm species including the date palm. In the coconut palm LY causes yellowing of the fronds, premature fruit drop and death of the tree in three to eighteen months. Mycoplasma-like organisms (MLO) were observed in the phloem of LY-diseased palms in Jamaica (Beakbane et al 1972, Plavsic-Banjac et al 1972, Heinze et al 1972), in Florida (Parsatharathy 1974, Thomas 1974, 1978) and in Africa (Dollet et al 1976, 1977). MLO distribution within the sieve element of the coconnut palm was found in the spear leaf, inflorescence, flag leaf and the root (Waters and Osborne 1978).

Remission of symptoms in palms was obtained by injection of tetracycline antibiotic in diseased trees in Florida (McCoy 1972, 1973, 1974, 1975, 1977) and in Jamaica (Hunt et al 1974). Absence of comparable control with other antibiotics suggests the causal agent to be an MLO. The disease was shown to be transmitted by an aerial vector (Carter 1966, Heinze 1972) although the vector was not identified. The incubation period of the disease in young palms was three to eleven months (Heinze and Schuiling 1973). Field studies by Dabek (1975) suggested that seasonal variations in temperature could affect MLO multiplication within palm tissue. Eden-Green (1976) described a correlation between coconut root necrosis and the foliar LY symptoms. In a comparative epidemiology assessment, it was calculated that rates of spread of LY were

the same in Africa, Jamaica and Florida (McCoy 1976); this pointed to the possibility of the same vector being involved in all three places.

An LY-resistant variety of coconut, The Malayan Dwarf, has been developed which is gradually being used to replace the dead trees in Jamaica and also in Florida (Harries 1970). However, this variety is reported to succumb to the disease in West Africa (Addison 1978), raising the question of new mutations of the pathogen that attack the resistant palm. Other varieties of coconut palm that are resistant to LY are being tested in Jamaica (Harries 1975).

Purpose:

Initially the purpose of this research mission was (a) to establish species of occasional palm feeders present on coconuts in Jamaica for comparison with results obtained in Florida (b) to identify possible vector species and test for survival on coconuts (c) establish life history of selected species (d) to test by aquisition vector status of species selected, and (e) to endeavor to transmit through vector injection the disease and produce evidence as to some of the transmission characteristics. Part (a) and (d) were undertaken simultaneously and a Physiological phenomenon in the lethal LY-diseased palms was also studied. Due to difficulties, enumerated in the 1st annual report, parts b, c, and e were ommitted from the research plan.

Preparations and Procurements:

Whereas the original research grant had no provisions for purchasing research equipments and supplies, and because there was insufficient research

materials available in Jamaica, the request was made and approved for additional funds of $5,200 from the Agency for International Development to satisfy the immediate research needs. These funds were used in 4 main areas.

1. Conversion of present facilities to a controlled-environment insect rearing room, and additions to greenhouse facilities in form of a lath-house for augmentation of host plants and insects.
2. Purchase of entomological field supplies such as a gasoline-operated insect collecting and sampling machine (D-Vac brand), screening materials for building field cages, plastics, tanglefoot, and light traps for catching insects.
3. Purchase of radioisotopes, X-ray films, proportional gas, modification of a nuclear counter in the University of the West Indies, and transportation costs of all above items.
4. Puschase of tools and applianaces for general wood, plastic, and metal work in shop, laboratory, and field.

Insects and Mites Transmission Tests

Mites:

Prior to discovery of mycoplasmas as causal agent of LY, a species of eriophid mite from the leaves of coconut was tested for possible transmission of LY (Heins and Schuling, 1971). In order to complete these tests we used a most abundant damaging mite on coconut fruits, *Eriophyes guerreronis*, for disease transmission. Young coconut fruits 4-5 cm in diameter were collected

from trees showing mite and LY damage. After leaving these fruits at room temperature for 36 hours, large numbers of adult and immature mites emerged and swarmed under the calyx, on the furit. These fruits were transferred to the field, in at ca 4° C in ice chest, where sections from under the calyx were excised and attached to healthy trees on 1) similar size fruits, 2) leaflets of the fronds, and 3) unopened spear leaves. Six trees were used in each of the 3 cases above. On each tree six sections (with mites confirmed to be present) were attached with tape. No transmission of LY was detected although the mature fruits showed evidence of extensive mite damage.

Insects:

In a series of experiments two different species of Empoasca from sweet potato and gongo pea plants were increased on these plants. Ca 1000 insects were collected by a D-Vac insect sampling machine, transferred to field at ca 4° C in ice chest, allowed to feed on diseased palms for 1 h, and were transported back to laboratory and maintained on potted host plants in the environmentally controlled rearing room. After 14 days the surviving insects were transported, on their host plants, to 5 x 5 x 4 m aluminum frame cages covered with 20 mest polyester screening containing four 2-4 year old palms and the following:

1. One transplanted diseased palm and several potted host plants with Empoasca.
2. Diseased parts of the palm in water and potted host plants with Empoasca; the diseased parts being the inflourescence, spear leaves, and fronds.

3. One host plant propagated with Empoasca breeding.

4. Both host plants propageted and both species of Empoasca breeding.

5. No vegetation or insects except the healthy palms.

No transmission was observed in these tests. The survival of Empoasca after aquisition feeding on the LY palms was 7-10%, and only about 2% by the end of the 2 weeks in the rearing room. There were about 1,000 Empoasca sp. used in each test, and although there were several re-introductions in each cage, the numbers were not sufficient enough to conclude that these insects could not serve as vectors.

Preliminary testing of two very abundant insects on coconut palms, Dawnaria sordidulum, and Apoganalia sp., was also done as possible vectors of LY in Irwine in December 1978. Thirty six D. sordidlum and 90 Apoganalia sp collected from LY diseased trees in Retreat were placed inside screened plastic cages on leaflets of healthy palms. There was no transmission prior to May 1979.

Homopterans of Coconut Orchards:

In a qualitative and quantitative survey of all homopterans attracted to light traps (LT) and yellow traps (YT), seven Jamaica Tall coconut groves around the island were sampled twice at 1-3 weeks intervals. Each sampling period lasted 5 days of continuous trapping by 16 YT, and 2 nights by 8 LT. Preliminary studies showed that most homopterans were attracted to LT during a 3.5 h period following sunset. The LT was therefore run ½ h before sunset to 3.5 h after. Each YT consisted of two 320

cm^2 pieces of yellow plexiglas 75 cm apart connected by a steel rod. The rod could be used for hanging from the tree or standing on the ground (Plate 2). The LT were the Star Beam toy lights (Burgess Co.) and consisted of 2-battery operated flashlight attached to a 0.91 m-long tube of translucent white plastic. Light tubes and the Yellow plastics were covered with a thin layer of a clear tanglefoot. Homopterans caught were collected with a small brush and placed in petroleum ether to remove the tanglefoot, then stored in alcohol for identification.

The LY sites sampled were Spring Garden and Caenwood in the parish of Portland, Plantain Gardens and Holland Estates in St. Thomas, and Retreat in St. Mary. The two healthy sites sampled were Drax Hall in St. Ann and Irwine in St. James. Most insects from Caenwood, Spring Garden, Retreat, Plantain Gardens, and Irwine have been tentatively identified by the author (Appendix). Those from other localities and the species unknown to the author are awaiting identification. The numbers and species of these insects, if determined seasonally, could be of value in the following areas:

1. Narrowing down the list of possible vectors to those species common in LY-diseased areas.
2. Confirms the seasonal and numerical distribution of the vector, if that species is among the species collected by our methods of trapping.
3. Contributions to knowledge of faunistic range of Homoptera.

Plate 2. A yellow trap, hanging from a 4-year old coconut palm.

Preliminary Studies with Radiotracer Methodology

A request was made by the author to participate in a training course, offered by FAO, International Atomic Energy Agency, and the Institute of Food and Agricultural Sciences, in Gainesville, Florida July 11-August 19, 1977. The International Programs office of the University of Florida, funded the authors' stay in Florida for the initial 3 weeks of instructions and laboratory work. Dr. G. Basham from Ft. Lauderdale joined me in Jamaica for 10 days in June 1978 and we used 35-S due to its low risk for the beginning experiments and slow decay allowing gathering of most of preliminary informations necessary about the behavior of isotope in the coconut palms.

Injections of S-35 in median fronds of two 4-year-old Jamaica Tall variety of coconut palms, and collection and analysis of the data (methods described in the next section of this report) (Appendix).
suggested the following:

1. Slow and uneven distribution of the S-35 in injected fronds.
2. Low distribution to other fronds of the palm.
3. Decrease of activity to the background levels in one week.
4. Shielding of much of radiation by the epidermis of the leaflets and the cuticle of the insects, reducing the detection.
5. A stronger radioisotope such as 32-P would be preferred.

Translocation in Lethal Yellowing Palms

Stuides of vascular tissues, translocation, and xylem pressure of lethal yellowing (LY) coconut palms have been reported in literature. In studies of dye uptake, carbohydrate distribution, and histology of LY coconut palms, Carter (1965) suggested that there was a wilting in coconut palm caused by xylem blockage at a point below the growing point. By observing uptake of injected dye in the stem, intake of solutions of different viscosities through stomata, and transpiration, Dabek (1973) concluded that upward translocation was reduced through an interference with transpiration due to stomatal closure. Recently, by the use of Scholander pressure chamber, the diurnal fluctuations in the xylem pressure was shown to be reduced, indicating a decreased transpiration (McDonough 1977). In our report the radioisotope 32-P is used to study this phenomenon in more detail in wet and dry seasons in Jamaica.

Methods and Materials

The movement of solutions throughout healthy and diseased palms was determined by injecting radioisotopes into the tree then sampling various tissues at timed intervals after injections. Radioisotope 32-P was dilluted with 1.0 M phosphate buffer solution at pH 7. Non-bearing young palms were used in Caenwood during the dry, and in Plantain Gardens during the rainy seasons. Diseased trees used in the dry season showed early LY symptoms; (0.5-1.5 yellow fronds and up to 25% spear leaf necrosis). Those used in the rainy season showed early or advanced symptoms (3-4 fronds dried or yellow and 100% spear leaf necrosis). In petiole injections, 1 ml of 32-P at specffied activities was injected 30-60 cm above the petiole base.

For stem injections, 1 mCi 32-P in 10 ml buffer was injected with a Mauget[R] Injector (J.J. Mauget Co., Burbank, CA) into the trunk of 5-year-old palms.

To measure root uptake of 32-P,4-year old palms planted in 104 ℓ drums were removed in dry season without damaging the roots. Soil was removed by placing the root mass in a rapidly running river for 24 h; then the palms were replaced in the drums containing 72 ℓ water with 1.3 mCi 32-P in 0.5 ℓ buffer mixture. In single-root uptake studies, the distal 6 cm of 2-3 mm diameter secondary roots from an early diseased and a healthy palm were dug up, excised, and were immediately soaked in water. These were dried with paper towels and were placed upright in 3 cc glass vials containing 1 cc of a mixture of 6^{-5} mCi 32-P buffer mixture. At intervals roots were removed, dried with paper towels, and stored until counting (4-7 h). The uppermost 1 cm sections, which never were in contact with radioisotope, were cut off and 32-P was determined in the gas flow counter.

To study downward movement (phloem), fronds were injected within 75 cm of the tip with 0.025 mCi 32-P in a 4-year-old diseased and a healthy tree. Samples were taken from medial and the basal leaflets below the injection points (1 and 2 m, respectively) at various time intervals as described.

Leaf samples were handled as described below. Terminal, medial and basal leaflets, or some combination of the 3 were separated from fronds at each sampling interval and carried the Central Radioisotope Laboratory at the Univeristy of the West Indies, Kingston. Leaf disks 1.5 cm diam were excised from the center of the leaflets and counted for 4 one-minute intervals. Means and standard deviations were computed. The cork borer used for cutting leaf disks was washed in detergent, rinsed in 100% ethanol and dried after

each sample.

The counting apparatus was a Nucleus Model 1100 Nuclear Analyser, modified to supply up to 3,000 electron volts (3 MeV) in increments of 200 Volts. This was used with a Nuclear of Chicago proportional gas detector Model D-47. Counting was done at 2 MeV with 50% amplification. Background readings obtained from empty planchetts and from nonradioactive leaf disks did not differ significantly.

Stomatal openings were determined on diseased and healthy palms by the silicon impression method of Zelitch (1967). These impressions were taken from each of two medial leaflets from one mid-age(medial)and one young (terminal) frond of each healthy, early LY, and advanced LY palm.. Samples were rated by measuring 100 stomates from each impression. Stomates with less than 2 micron transverse separation between guard cells were rated as "closed", all others were rated as "open".

Results and Discussions

When bases of healthy palm petioles were injected with 32-P, significant activity was detected in distal leaflets on the frond at the first sampling period (4 h in dry season, 30 min in wet season)-- in trees with LY, this transport was absent or greatly reduced (Fig. 1, Table 1). Transport was almost completely inhibited in early stage LY palm in dry season except in the youngest expanded frond; transport even in the young frond was at a rate almost 20 fold less than in the healthy control (Fig. 1). In wet season, when water for xylem transport was more readily available, transport in an early LY palm was higher than that in the dry-season LY palm (Table 1, Fig. 1) but was still only about half that observed in the healthy control. Further, the initial 32-P transport in palms in early stages of LY was greater than

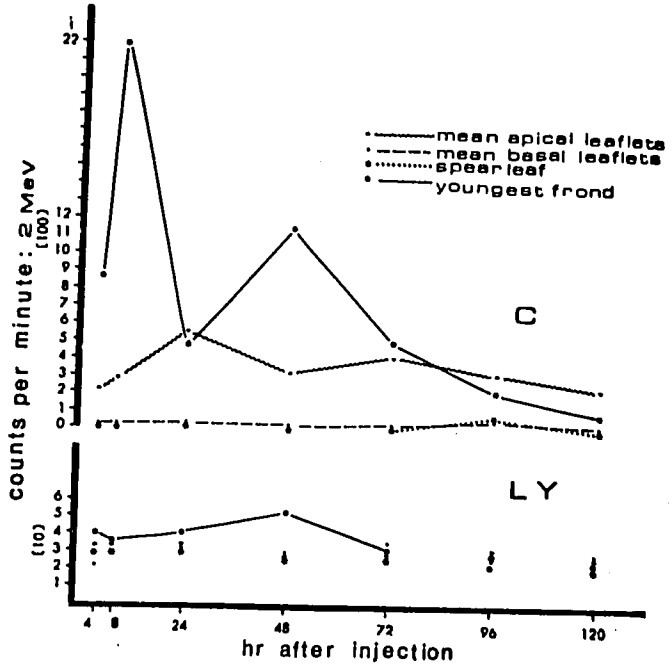

Fig 1. Upward translocation of 32-P injected in petiole bases of 4-year-old Jamaica Tall variety of coconut palm in the dry season in Jamaica except the spear leaf: an early stage lethal yellowing-diseased (LY), and a healthy (C) palm. All connected points indicate counts above background (27-33 CPM).

Table 1. Relation of intensity of lethal yellowing disease and the translocation of 32-P injected in coconut palm frond petioles in rainy season, May 1979, Jamaica: counts per minute 1/ from one leaflet disk per frond at 2Mev.

fronds yellow or dried/ % necrosis of spear leaves	Hours after Injection						
	0.15	1.00	2.00	4.00	8.00	24.00	120.00
0/0 (control)	430±70	5458±124	2984±96	1905±133	1271±31	1042±26	NR 2/
0.5/5%	144±17	131±13	NR	132±15	168±15	280±32	642±11
1.5/10-25%	27± 5	62± 4	125±10	1154±10	NR	1305±22	NR
3.5/100%	36± 5	33± 3	36± 6	34± 4	33± 6	33± 2	51± 3

1/ CPM: Mean of 4 one-minute counts ± standard deviation-- background = 30-38 CPM.

2/ No reading

that of palms showing advanced symptoms (Tables 1 & 4).

When healthy and LY palms were injected in the trunk with 32-P, activity was detected in fronds of healthy trees within 4 h (1st reading) after injection. No actibity was detected in fronds of LY palms. When 32-P was supplied by water through root feeding of whole trees in the drums activity was detected within 24 h in healthy palm fronds and was not detected after 5 days in fronds on an early LY palm (Fig. 2).

Since physical alterations, decline, and necrosis of root system in coconut trees with LY symptoms have been reported by other workers (Carter et al 1965, Eden-Green 1976), we investigated whether blockage of transport in root-uptake experiments was due to impaired root function or to some malfunction at another site in the palm. We examined uptake of 32-P in excised roots from diseased and healthy palms. Uptake by roots of healthy trees was 2-4 x that in roots from LY palms (Table 3). If the reduced uptake of 32-P at the roots reflects a loss of ability of the roots to take up water, root damage in LY palms would contribute significantly to the reduced xylem transport observed.

McDonough (1977) has shown that xylem water tension in leaves of palms with LY is relatively low. This suggests that water supplies are available in fronds to allow some transpiration and water transport in the leaves of diseased palms. Yet we observed greatly reduced transport of 32-P in palms in which petioles ere injected with radioisotope (thereby by passing the restriction of reduced root uptake of 32-P). In an attempt to resolve this point, measurements were made of stomates on diseased and healthy palms (Table 4). The data indicate that the evaporation area available to provide the motive force for water (% stomates open- see Zelitch, 1961) is reduced as LY symptoms advance in the palms.

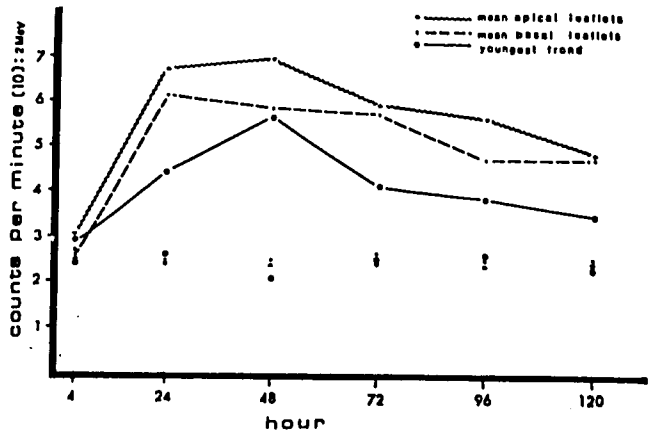

Fig. 2. Root absorption of 32-P in 4-year-old Jamaica Tall variety of coconut palms in the dry season in Jamaica: an early stage LY diseased, and a healthy palm (connected points)--background, 24-27 CPM).

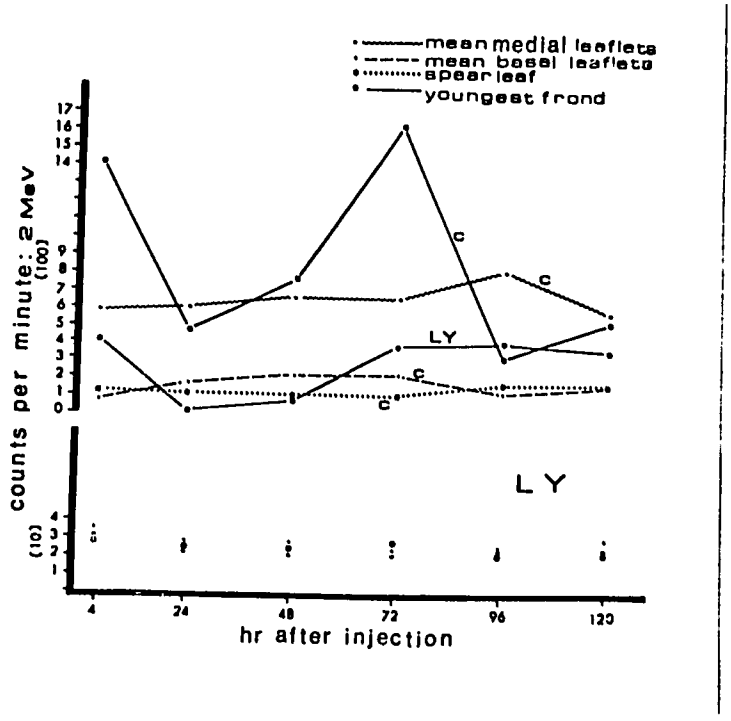

Fig. 3. Downward translocation of 32-P injected in rachis tips of 4-year-old Jamaica Tall variety of coconut palms in the dry season in Jamaica except spear leaf: an early stage lethal yellowing-diseased (LY), and a healthy (C) palm. All samples taken below the injection point. All the connected points indicate counts above background (19-28 CPM).

Table 2. Relation of occurance of radioactivity in leaflets of 32-P injected coconut fronds and in *Haplaxius crudus* feeding on it.

min after injection	CPM 1/ in leaflet	# radioactive insects
	Healthy Tree	
30	71±6	4
60	179±10	2
90	199±17	3
120	227±10	5
150	280±19	6
	Lethal Yellowing Tree	
30	34± 5	0
60	27± 5	0
90	54± 7	1
120	125±10	1
150	435±20	2

1/ Count per minute: 4 one-minute counts from a leaflet disk ± standard deviations -- background = 30-38 CPM.

Table 3. Uptake of 32-P by excised secondary coconut roots in vials during rainy season, May 1979, Jamaica: Counts/min ± standard deviation[1/]

Trees	Minutes Exposed to 32-P				Mean[2/]
	10	20	30	60	
Healthy	88±82	192±215	241±366	441±410	210
LY-diseased	64±31	51± 5	170±116	112±116	99
Mean[2/]	76	121	205	276	

1/ Background: 38±4 CPM

2/ F values significant for healthy & diseased (68.88), times of exposure (56.65), and interaction of trees vs time (324.58).

Table 4. Relation of translocation of 32-P [1/] and open stomata in 4-year-old coconut trees in wet season, May 1979, Jamaica.

Location of the frond	Healthy		Early Ly-diseased [2/]		Adv. LY-diseased [2/]	
	CPM [3/]	% stomata [4/]	CPM	% stomata	CPM	% stomata
Medial	528±176	82±13	565±345	53±6	31±3	2±2
Terminal	2087±1805	86±15	527±802	38±9	36±6	0

1/ $\bar{x} \pm s$ for CPM at 0.5, 1, 2, 4, 8, 24, 48, and 120 h after injection.

2/ See text

3/ Count Per Minute; background, 29±4 CPM.

4/ 6 days after injection at 2:30 PM in shade.

These data confirm the reduction of xylem transport in palms with LY. The data is consistant with the hypothesis that the reduction in transport is due to both root malfunction and by abnormal stomatal behavior. Further information will be necessary before the mechanism of the reduction and the relative importance of root vs leaf affects can be determined.

Since the mycoplasma which apparently cause lethal yellowing are seen only in the phloem of diseased palms (Parthasarathy 1974, Thomas 1979), we tested the effect of LY on phloem transport. When 32-P was injected into the distal end of the rachis in a healthy palm, activity was exported from the frond (phloem transport) and detected in the uninjected spear leaf. No such export was detected in an early-stage LY palm (Fig. 3). This however, is not clearcut evidence for inhibition of phloem function. It is possible that the injected 32-P was not moved from the injection sight to the leaflets, at the extreme top of the frond, in the diseased palm due to reduced xylem transport and, therefore, was not loaded into the phloem. Thus the lack of phloem transport in this experiment is equivocal. In a second experiment a homopteran insect Haplaxius crudus which feeds on the phloem sap of coconut was allowed to feed on healthy and diseased palms which had been injected with 32-P. Insects acquired isotope from healthy and diseased palms after isotope had moved into the region where insects were feeding (Table 2). In diseased palms, however, fewer insects acquired activity than in healthy palms. The mean number of insects labeled per mean detectable CPM in leaf tissue, over all time periods (Table 2), is 2.1×10^{-2} insects/CPM in healthy tissue and 0.6×10^{-2} insects/CPM in diseased trees. Those data are consistent with the hypothesis that some aspect of phloem function is altered in diseased palms. On the other hand these observations could merely reflect a reduction

in ingestion of phloem sap by insects feeding on diseased palm rather than reduced loading on transport of 32-P in phloem.

Data indicate that 32-P is loaded into the phloem in healthy trees. First, after the initial rapid distribution of 32-P in the xylem, there is slower export of 32-P against the direction of xylem flow, moving out of the injected frond and into the rapidly growing spear leaf. This pattern of movement indicates phloem transport. Second, H. crudus, a known phloem feeder (Waters 1977), acquires 32-P when allowed to feed on labeled palm leaflets.

Work with diseased palms will be more difficult because the xylem disfunction (and a possible phloem disfunction) observed in such palms prevents ready transport of material to the phloem, where it is necessary to use this technique. To detect insects feeding on diseased palms, it will be necessary to: 1) use young leaves from palms in early stages of LY, 2) use relatively high amounts of 32-P, 3) make actual determinations of 32-P in the leaflets of test palms to insure that label is present at the time of experiments.

This study has shown that petiole injection in healthy palms is the best way to get sufficient radioisotope into palm leaves for experiments designed to identify candidate insect vectors of lethal yellowing.

Homopterans Feeding on Coconut Palm

Although several species of Homoptera have been found on coconut palm, or have been kept alive for several days in cages on palm (Carter 1966, CIB Res. Rept. 1968-77), there has been no systematic study of the homopterans feeding on coconuts. Such a study was initiated to identify species of the Homoptera which had the potential to vector LY agent in Jamaica

The radioisotope 32-P was injected into 45 Jamaica Tall variety coconut palms Jan 15-May 25, 1979 in two LY locations, Caenwood and Plantain Gardens, in the dry and rainy seasons respectively. Approximately 10,000 homopterans were trapped on/and around these trees and checked for ingestion of radioactivity using X-ray film and gas flow counting techniques. Trees were tagged by injection with 0.05-0.3 mCi $Na_2\ ^{32}P\ PO_4$ in a stock solution of 0.1 M phosphate buffer, PH 7. Light traps and the yellow traps (described in the previous section) were hung from the trees or fixed in the ground on or near the injected trees. Green polyester 20 mesh screens reinforced by steel wires made the cone traps which allowed insects access to the crown of the tree, but restricted their exit, used on some radio labeled 2-year-old Jamaica Tall variety of coconut palms. The insects were captured on sticky cone inverted inside a 2 lit.screened polyethelene container positioned over the exit port of the upper cone (Plate 3). The traps were inspected daily and the Homoptera were collected with a brush or spatula, placed directly on an 8 x 10 clear sheet of plastic, covered with a thin plastic wrapper, and placed face down on X-ray film for 3 days. Those specimens showing activity on the film were examined on the 4th day by a Nucleus Model 1100 counter, modified to supply up to 3 Mev with interval controls of 200 volts and attached to a Nuclear Chicago Model D-47 proportional gas detector. Twenty two species were tentatively identified and along with 7 genera were

recorded as having fed on the palms injected with 32-P. Those identified with their numbers, location, date, autoradiographis intensity, and counts per minute were recorded (Table 5).

In other experiments insects were collected with a D-Vac insect collecting machine from the underbrush, borders of orchards, and native vegetation in Caenwood and Spring Garden. The homopterans were confined for 8 h in screened clear plastic cages enclosing 2 leaflets on each of the 3 fronds injected with 0.5 mCi of 32-P (Plate 4). Those species which acquired radioactivity by this method, were determined after being killed by ether and being placed on x-ray films for 3 days (Table 6).

All identifications are tentative, using the available keys and the museum specimens in Jamaica. Final publication of the results await the positive identification by taxonomists of the particular groups. Experimental controls confirmed that acquisition of radioactive was not due to surface contaminations of insects by contact with radioactive palms. A few insects which were not phytophagus showed radioactivity. In cone traps ants which may have fed on honeydew of palm aphids in the spear leaf were captured. Radioactive houseflies also caught in cone traps may have lapped the sap from the freshly cut fronds too large to fit under the cones. When several insects of different orders were confined to 2 leaflets of a highly radioactive frond for 8 h, Hippelates sp. showed radioactivity by autoradiography. These adults of the eye gnat feed on animal and human mucos secretions, nectar from plants, and are capable of scarifying animal mucos membranes and pericarp of such dried fruits as prunes with comb shaped ridges on the labrum (M.S. Mulla, personal communication), thus they may well be capable of breaking the leaf surface and ingesting plant sap in search of moisture in confinement.

Plate 3. A 3m-high cone trap, made of steel rings, wooden braces, and 20-mesh green polyester screen, covering a 2-year old coconut tree.

Plate 4. A 30-cm clear screened plastic cage confining insects on leaflets of a frond of a coconut palm.

Table 5. Homopteran insects naturally fed on coconut palms (Cocos nucifera L.), tagged with P-32, and attracted to traps in Jamaica during a dry(Jan., Feb., Mar.), and a wet(Apr., May) season in 1979.

Insects		Trap				Counts/minute 4/		Previous reports		
name	no	type 1/	Place 2/	date	film 3/	insect	bkgd	Puerto Rico	Florida 5/	Jamaica 5/
Agallinae	1	YT	C	Feb	2	23±5	22±6			
Agalliopsis tropicalis	1	YT	PG	Jan	1	33±4	28±4			
Apoganalia sp.	1	LT	C	Feb	2	48±5	24±2			7/
Apoganalia sanguinipes (V.D.)	1	LT	PG	Jan	1	34±6	28±4			7/
Antillixius sp.	1	YT	PG	Feb	1	41±5	24±2			8/
Bothriocera cotiso (Fenn.)	1	CT	C	Feb	1	33±5	24±2		9/	10/
Cedusa sp.	8	YT,LT	PG	May	1-7	30-157	21-39		11/ 9/	
Cedusa wolcotti(Muir)	8	YT,LT	PG	May	1-4	38-125	35-38	14/	9/	
Chlorotettix sp.	2	LT	C	Jan,Feb	1	19-26	12-22		11/	10/13/12/
Colpoptera maculifrons maculata Dozier	1	YT	PG	Jan	1	35±10	31±1	14/		
Dawnaria sordidulum (Muir)	26	Asp,LT,YT,CT	C,PG	Jan-May	1-7	24-235	22-38	14/		
Deltocephalus flavicosta Stal	3	CT	C	Feb	1-2	26-28	22±2	14/	11/	10/
Empoasca sp.	11	LT,YT	C,PG	Jan-May	1-3	19-52	17-38	14/	11/	8/
Haplaxius crudus (Van Duzee)	10	Asp,YT,LT,CT	C,PG	Jan-May	1-4	20-190	9-38		11/ 9/	8/ 7/
Hortensia similis (Walker)	1	CT	C	Feb	3	159±4	24±5	14/	11/	10/12/
Idioderma varia (Van Duzee)	4	LT	C,PG	Feb-May	2-3	23-44	22-38			
Idioderma sp.	1	YT	C	Feb	2	27±6	25±3			
Joruma apicata n sp.	1	YT	P	Jan	1	30±3	28±4	14/		
Metascarta histrio	1	?	P	Jan	1	27±4	23±4			12/
Nilapravata wolcotti (Muir & Giffard)	1	LT	P	Jan	1	32±2	28±4	14/	11/	
Nesosteles lineata (Osborn)	1	LT	P	May	2	43±6	28±5			

Table 5. (continued)

Insects name	no	Trap type 1/	Place 2/	date	film 3/	Counts/minute 4/ insect	bkgd.	Previous Reports Puerto Rico	Florida 5/	Jamaica 6/
Omolicna (puertana ?) cubana	22	Asp,CT,LT,YT	C,PG	Jan-May	1-7	17-215	12-38	14/	9/	8/10/
Omolicna sp.	5	Asp,YT,CT,LT	C,PG	Feb-May	1-2	28- 61	20-38			
Olarius sp.	1	?	PG	Jan	1	23±5	12±2		11/	10/
Osbornellus bimarginatus (DeLong)	5	CT,YT	C	Jan-Mar	1	18-34	9-23	14/		7/
Punona magna	1	YT	C	Feb	1	26±3	21±2			
Sanctanus fasciatus (Osborn)	1	?	PG	Jan	1	19±3	12±2	14/	11/	10/
Saccharosydne saccharivora (Westwood)	2	YT,LT	C,PG	Feb,May	2,3	25,36	22,35	14/	11/	10/
Typhlocybella maculata n. sp.	1	YT	C	Feb	4	25±3	22±3	14/	9/	10/13/
Tylozygus fasciatus (Walker)	2	Asp,LT	C	Feb	2,3	24,26	21,22	14/		13/
Xestocephalus tessellatus	4	CT,LT	C	Feb-Mar	1	21-27	9-26		11/	10/13/

1/ YT, yellow trap; LT, light trap; Asp, aspirator; CT, cone trap-- traps underlined caught the most
2/ C, Caenwood; PG, Plantain Garden
3/ Autoradiograph, given a visual index of intensity 1-7.
4/ At 2 MeV after 3 days of autoradiography, showing the range among different individuals or the mean and the standard deviation for one insect -- bkgd= background
5/ Document at University of Florida Agr. Res. Cent., Ft. Lauderdale, FL 33314.
6/ Document at Coconut Industry Board, P.O. Box 204, Kingston 10, Jamaica.
7/ A.J. Dabek, personal communication
8/ Johnson and Schuling 1974,
9/ Howard, F.W., and F.W. Mead 1979.
10/ Carter, W. 1965
11/ J.H. Tsi, personal communication
12/ S. Eden-Green, personal communication
13/ Heinze and Schuling 1971
14/ Caldwell and Martorell, 1956

Table 6. Homopterans which fed in plastic cages on leaflets of 2-4 year-old coconut trees injected with 32-P, among those collected by D-Vac insect sampling machine from undergrowth near the coconut groves, April 1979.

Name	no.	Film 1/
Agaliopsis tropicalis	11	1-10
Cicadulina tortilla n.sp.	3	2-3
Colpoptera rugosa	3	1-3
Deltocephalus flavicosta Stal	2	1-2
Dikranura albatron n.sp.	2	1-3
Empoasca sp.	2	1-2
Hortensia similis (Walker)	10	1-10
Omolicna (*puertana*) *cubana*	1	1
Tylozygus fasciatus (Walker)	6	1-2
Typhlocybella maculata n.sp.	1	2
Typhlocybella minima Baker	25	1-10

1/ Exposed area on x-ray film autoradiograph, given an intensity index range of 1-10

The naturally fed homopterans (Table 5) vary considerably in their numbers caught in traps and the amount of radioactivity detected in them. Those having few CPM may not have necessarily ingested less sap. The differences in size of very small cicadellids such as Typhlocybella, Empoasca, Joruma, and Nesosteles with large fulgorids such as Punona, Apoganalia, Colpoptera, Dawnaria, and Haplaxius, combined with 3 days delay due to screening of large number of insects by autoradiography, may be the reason for low autoradiograph intensity and CPM, respectively. On the other hand confining insects to the tagged palm may have caused forced feeding of some species (Table 6) normally not feeding on coconut under natural conditions, or feeding less, although it confirms some insects of Table 5.

Considering that 32-P has a physical half-life of 14.28 days, and in the field it rapidly translocated to other parts of the palm unavailable to the foliage feeding homopetrans (Fig. 4), in view of the few number of trees tagged for environmental protection reasons, a surprisingly high number of species which fed on palms were captured. This indicates that a quantitative study of the feeding behavior in the field can be done, using our methods, once the vector is discovered. Some of the species of Tables 5 & 6 have been poorly tested for LY transmission, or not have been tested at all. Such are Dawnaria sordidulum, Idioderma varia, various species of Empoasca, Typhlocybella minima, T. maculata, Cedusa wolcotti, other unidentified species of Cedusa, and Agalliopsis tropicalis. The feeding habits of most species in Tables 5 & 6 are unknown. An easy test of labial imprints (Waters 1977) can determine their phloem or xylem feeding habits. We recommend at least the phloem feeders of these insects be tested in future LY MLO vector transmission studies in Jamaica.

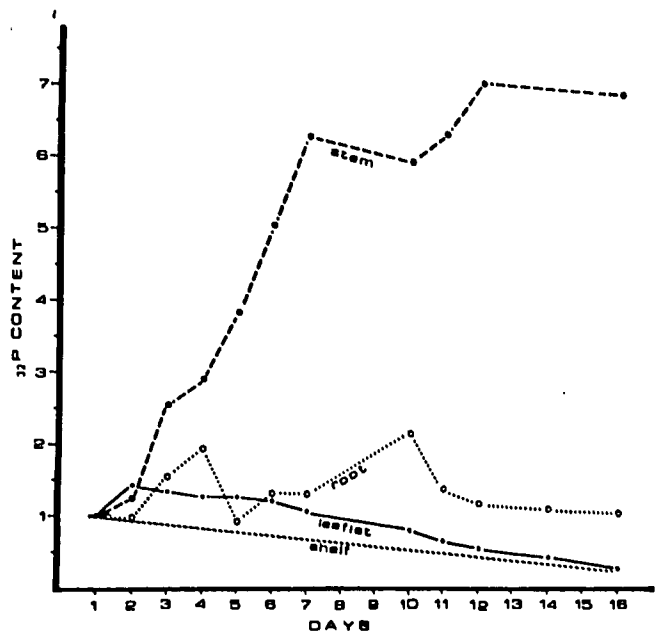

Fig. 4. Movement of 32-P in a Jamaica Tall variety coconut palm injected with 0.3 mCi in each 8 petioles of the fronds.

$$\text{Content of 32-P} = \frac{\text{Count per minute (CPM) of each day after injection}}{\text{CPM of the 1st day}}$$

Appendix

A partial list of some homopteran insects from coconut groves in Jamaica, caught in tanglefoot on light traps operated 8 h (4 h/night) and on 16 yellow traps and 6 cone traps operated 120 h, on each date.

Insect	no
Spring Garden, 3/15/78, cone traps	
Bothriocera sp.	1
Chlorotettix sp.	6
Empoasca sp.	21
Haplaxius sp.	2
Hortensia sp.	1
Olarius sp.	1
Osbornellus sp.	5
Spangbergiella sp.	1
Caenwood, 9/1/78 & 9/4/78, Cone Traps	
Agalliopsis tropicalis	2
Balclutha sp.	1
Chlorotettix sp.	13
Empoasca sp.	24
Haplaxius crudus	2
Osbornellus bimarginatus	2
Sogatodes cubanus	1
Spangbergiella vulnerata	1
Tangella sp.	1
Xestocephalus tessellatus	1
1 unidentified species	

Appendix

(continued)

Insects	no
Caenwood, 9/5/78 & 9/13/78, light traps	
Apoganalia sanguinipes	33
Balclutha sp.	17
Colpoptera sp.	2
Empoasca sp.	2
Haplaxius crudus	1
Hortensia similis	1
Nesosteles sp.	2
Osbornellus bimarginatus	2
Punona insularis	1
Peregrinus sp.	1
Protolebra braziliensis	1
Sanctanus sp.	1
Sogatodes approximata	1
Tylozygus fasciatus	1
Typhlocybella minima	1
Xestocephalus maculatus	1
Xestocephalus pulcarius	2
Xestocephalus sp.	6
3 unidentified species	
Caenwood 9/5/78 & 9/20/78, yellow traps	
Agalliopsis tropicalis	17
Apoganalia sp.	29
Balclutha sp.	1

Appendix

(continued)

Insects	no
Caenwood .. (continued)	
Calicentrus sp.	1
Cibovia coffeacola	1
Colpoptera sp.	43
Dikranura sp.	1
Empoasca sp.	39
Hortensia similis	8
Haplaxius crudus	1
Idioderma sp.	3
Metascarta histrio	10
Nilapravata wolcotti	2
Peregrinus maidis	10
Scaphytopius fuliginosus	2
Typhlocybella minima	8
1 unidentified species	
Montego Bay (Irwine), 10.11.78 & 10/25/78, light traps	
Apoganalaia sp.	8
Balclutha sp	5
Cicadulina tortilla	1
Deltocephalus falvicosta	2
Empoasca sp.	1
Graminella cognita	1

Appendix

(continued)

Insects	no
Montego Bay . . (continued)	
Typhlocybella minima	2
Xestocephalus tesselatus	1
Xestocephalus sp.	1
2 unidentified species	
Montego Bay (Irwine), 10/8/78 & 10/25/78	
yellow traps	
Agallia pulchra	1
Agallia configurata	1
Agalliopsis tropicalis	5
Chlorotettix sp.	1
Cicadulina tortilla	6
Colpotrera sp.	25
Empoasca sp.	4
Hortensia similis	1
Idioderma sp.	3
Peregrinus maidis	1
Poeciloscarta histrio	1
Protalebra tabebuiae	1
Protalebra sp.	2
Scaphytopius fuliginosus	3
Typhlocybella sp.	1
Tylozygus faciatus	1
10 unidentified species	

(continued)

Insects	no
Plantain Gardens, 11/3/78 & 11/9/78, light traps	
Chlorotettix sp.	1
Cicadulina tortilla	4
Cubana sp.	1
Deltocephalus flavicosta	5
Empoasca sp.	5
Graminella cognita	2
Haplaxius crudus	2
Hortensia similis	1
Nesosteles incisa	13
Nesosteles liniata	5
Nesosteles sp.	2
Omolicna cubana	4
Omololicna sp.	1
Tylozygus fasciatus	2
Xestocephalus tesselatus	1
Xestocephalus sp.	1
Retreat, 12/4/78, yellow traps	
Haplaxius crudus	2
Idioderma sp.	2
Nesosteles incisa	1
Oliarus complectus	29
Omolicna cubana	7
Peregrinus maidis	2
1 unidentified species	

Appendix

Counts per minute from lower surfaces of the disks from the basal and terminal leaflets of 4 fronds each injected with 0.4375 mCi 35-S, and from those of the axial frond on the same tree.*

Time after inject.	Mean g/ 2 discs	Fronds 1 basal	1 terminal	2 basal	2 terminal	3 basal	3 terminal	4 basal	4 terminal	Axial leaf basal	Axial leaf terminal
4 h	----	61±10	452±33	112±7	403±30	146±10	4863±254	68±11	1315±34	165±19	----
8 h	0.0352± 0.0030	331±19	205±10	49±14	634±59	318±36	1324±31	38±8	152±18	45±9	556±18
12 h	0.0367± 0.0034	41±12	192±22	52±13	88±17	473±32	1427±83	442±38	341±21	56±7	482±30
16 h	0.0372± 0.0036	680±28	674±23	542±18	598±50	53±8	2835±113	48±10	227±19	54±3	47±10
20 h	0.0383± 0.0037	56±13	631±20	346±24	118±13	56±8	1006±55	53±13	687±28	56±12	51±8
24 h	0.0366± 0.0033	49±8	586±23	44±9	659±19	44±11	608±18	535±47	233±28	54±12	547±44
36 h	0.0370± 0.0045	335±21	237±22	87±9	364±9	55±5	954±10	426±20	78±11	48±5	444±30
48 h	0.0368± 0.0033	462±12	709±38	48±2	507±21	51±4	614±13	429±14	540±5	207±27	47±15
3 days	0.0387± 0.0031	42±7	1171±83	189±13	133±5	54±6	1366±48	45±8	98±5	170±17	48±8
4 days	0.0379± 0.0024	53±5	1335±83	56±8	115±9	52±8	1060±28	48±9	306±14	59±11	55±8
5 days	0.0340± 0.0030	58±8	865±36	47±13	89±12	62±7	356±25	52±9	94±12	60±5	51±12

* Background: 24th h, 42±9; 3rd day, 43±9 ; 5th day 49±10

Counts per minute from median leaflet discs of all the coconut fronds 6 days after injection with 1.75 mCi 35-S in the tree (disc mean weight; 0.0448±0.0051)*

1	2	3	4	5	6	7	8	9	11	12	13	14	15
38±5	38±2	37±9	39±9	38±11	44±8	83±7	50±8	227±17	64±13	49±6	45±11	44±3	51±10

* Background: 44±7

Appendix

Counts per minute of the fresh upper and lower surfaces, and from the air-dried lower surface of the coconut leaflets severed 4 h after injection with a 1.75 Mci 35-S.*

Surface	g mean weight 2 discs	Samples						
		1	2	3	4	5	6	7
Fresh upper	0.0697± 0.0149	375±16	333±7	72±10	3084±128	50±30	1315±35	63±7
Fresh lower	0.0697± 0.0149	452±33	403±30	146±10	4863±254	68±11	1435±27	61±10
Dried lower	0.0251± 0.0071	502±45	98±14	33±5	10320±342	41±7	2659±70	53±8

* Background: 42±9

Appendix

Count per minute from each of the leaflet discs taken distal and proximal to the rachis of the 4 fronds 48 h after injection with 0.4375 mCi 35-S each. 1/

	BD	TD	BP	TP 2/
Mean g weight/ disc	0.0161± 0.0026	0.0132±	0.199± 0.0036	0.0216± 0.0008
Frond 1	499±15	352±23	460±20	517±22
Frond 2	55±9	494±56	56±7	498±36
Frond 3	40±9	286±34	54±12	472±19
Frond 4	501±18	111±18	470±32	581±19

1/ Background: 49±10

2/ BD: disc taken distal to the rachis on the basal leaflet

TD: " " " " " " terminal "

BP: " " Proximal " " " basal "

TP: " " " " " " terminal "

Appendix

Mean disintegration per minute from insects confined 24 h to terminal leaflets of coconut frond with radiosulfur activity of 1335±83 DPM

40 *Chlorotettix* sp	56±3
" " mashed	62±5
38 *Sanctanus* sp	62±5
" " mashed	120±5
30 *Empoasca* sp	52±9
" mashed	
Background	39±5

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