https://doi.org/10.29321/MAJ.10.A04104

Madras agric. J., **45** : (3) 89—98, 1958.

A *Pythium* Root Rot and Chlorosis Complex of Sugarcane

by

K. V. SRINIVASAN

Sugarcane Breeding Institute, Coimbatore

During the last two years a disease of sugarcane has been observed in the neighbourhood of Coimbatore. The chief symptom is a marked and severe chlorosis. Affected plants may be recognized from a distance by the striking white colour of the crown leaves. The disease has also been noted in some varieties in the collection of this Institute. It has been observed on Co. 419, Co. 421 and Co. 467 at Tudiyalur north of Coimbatore. Specimens of Co. 419 forwarded from Pugalur in the Tiruchirapally district, and examined through the courtesy of Dr. K. Ramakrishnan also showed similar symptoms. At Coimbatore it has been so far observed only in the gardenland soils but not in wetland soils. In the collection at the Institute the disease is not confined to any particular species of *Saccharum* but occurs in different species.

Symptoms: There are atleast three distinct phases in the life history of the sugarcane plant when the disease appears. Germination failure is common in the planted setts. This is quite distinct from the 'pineapple' disease caused by *Ceratocystis paradoxa* (deSeynes) Moreau. '*Spontaneums*' and certain '*officinarums*' are particularly prone to such failures. Either the shoot does not emerge at all or if it emerges the shoot is sickly, yellow or almost white and soon starts withering. The sett may easily be pulled out of the soil. There are no sett roots or if a few have developed they bear a number of red lesions, the root tips are blackened and the roots are in various stages of decay. The sett may be slimy and the tissues are of a red colour and emit a disagreeable odour. They do not however emit the smell of over-ripe pineapples characteristic of 'pineapple' disease. The sett soon undergoes a wet rot and disintegrates.

Some setts may germinate normally and the first leaves may be green and healthy looking. But in two to six weeks the leaves turn yellow and soon turn almost completely white. Short red longitudinal streaks appear on the leaves. The plant remains severely stunted and may wither away in a few weeks or carry on an unthrifty existence. On digging out the plants the sett roots and the newly formed shoot roots exhibit pits, scattered reddish lesions and root tip injury and are rotted back. In certain varieties like Co. 419 recovery may occur after 8 to 12 weeks when new green leaves are developed and the chlorotic leaves wither away.

The disease may also occur after cane formation at any time from four months onwards up to maturity. The leaves of one or more tillers may begin to exhibit alternate green and yellow stripes all along their length. Later leaves show progressively increasing chlorosis till the top leaves are bleached white (fig 1). In such completely chlorosed leaves short narrow red streaks may appear along the length of the blades. The sheath does not appear to be affected. The oldest leaves may not show the symptoms. Late tillers bear a crown of white leaves and none of the leaves on such tillers may be normal. The root system shows extensive injury. Many of the roots show reddening of the ends and bear blackened root caps. Many pits and red lesions are seen all along the length. The lesions are circular or elliptical and of varying size, mostly three to five millimeters in diameter. Some of the lesions may be of a darker colour. The branch roots, secondaries and tertiaries, are rotted back beginning from the tip nearly up to their points of origin. Often only a cluster of long primary roots almost devoid of branches is seen. Sometimes there may be a cluster of branches at the end of a primary root and the rest of the root may not bear any living secondary roots. The anchorage of the plant does not appear to be seriously affected at this stage.

Sections through the root lesions at various stages of the disease reveal that the cortex is injured. In older primary roots the stele is not affected although the cortex may have suffered extensive injury and may slough away. However in very young roots almost the entire root is destroyed. Embedded in the tissues especially in recently affected roots may be seen a number of nematodes in various stages of development. In older lesions however few nematodes may be seen, presumably because the latter have deserted the roots. Fungal mycelium occurs both intra and intercellularly in the affected tissues.

Aetiology: Examination of the roots and the rhizosphere showed the presence of a large number of nematodes. On certain roots there were hundreds per linear inch of root. The nematodes were separated by the Baermann funnel technique. In the beginning a number of forms were encountered many of which were undoubtedly saprophytic. By repeated transfer of washed and chopped roots to fresh plants raised on sterilized soil the pathogenic form was obtained in a relatively pure form. The separated worms were fixed in hot water and 5% formalin and examined. The females were predominant. These are 0·6 to 0·7 mm. in length and 20 to 29 μ in diameter; oesophagus 90 to 100 μ, tail 70 to 75 μ, vulva posterior to the middle about 60 μ distant; there are rather coarse striations on the cuticle. The body has crenate winged edges at the posterior end and the tail tapers to a blunt conical apex. The general appearance of the worms suggests their similarity to *Radopholus similis* (Cobb) Thorne (=*Tylenchus similis* Cobb, *Anguillulina similis* (Cobb) Goodey).

The worms on separation were washed by centrifugation in sterile water and plated on oatmeal agar. The cultures were characterised by their slimy appearance. Within 5 to 8 weeks the cultures contained numerous worms. A species of *Fusarium* belonging to section *elegans* was associated with the cultures and the worms did not thrive in the absence of the fungus in the culture. In the nematode cultures the *Fusarium* produced submerged growth but when purified by single spore technique it produced whitish aerial mycelium and salmon coloured pionnotes.

Isolations of fungi were made from the root rot lesions by plating roots on plain agar after several washings in sterile water and transferring hyphal tips of the resulting growth to Quaker oats agar. In all cases a species of *Pythium* was obtained. Hyphae are hyaline, coenocytic, with granular contents, 4 to 7 μ in diameter; sporangia formed of inflated segments and irregular toruloid digitate elements at the ends of branches or in intercalary fashion; zoospores 20 to 40 per vesicle, 8 to 10 μ in diameter when encysted; oogonia readily formed on all media and in the tissues of the host, spherical, thin-walled, smooth, terminal, 20 to 35 μ in diameter; antheridia monoclinous, 1 to 4 per oogonium, clavate; oospores plerotic, smooth-walled, yellowish to brown 18 to 32 μ in diameter. In certain tubes of quaker oats agar chlamydospore like bodies were formed after about a month which germinated by germ tubes. The organism appears to be identical with *Pythium graminicolum* Subram. (Middleton, 1943).

Pathogenicity: Pathogenicity was studied with Co. 467, which proved to be highly susceptible to the disease, as test variety. The results are given in table 1. Coimbatore local, a variety of *spontaneum* was also used principally for studying germination failures.

(i) *Nematodes:* Single-eyed cuttings were planted in soil taken from affected fields and sterilized by autoclaving. At the time of planting, nematodes were added to the soil as a suspension made up by mixing an agar culture with water.

The setts of *spontaneum* failed to germinate and on digging out were found to have decayed. The root-eyes were damaged and no roots had developed. The bud was also soft and rotten. The internodal tissue was soft and slimy.

Nematodes were found to have colonized the setts in large numbers particularly the root eyes and buds.

In the case of Co. 467 the shoots emerged. In 9 out of 20 cases the leaves showed typical chlorosis. The roots had pits all over the surface and slight reddening around the pits. There was no rotting of the roots. Isolations yielded, besides the nematodes, *Fusarium* sp. usually found associated with the nematode cultures.

(ii) *Fusarium sp.:* The *Fusarium* was multipled on sand-oats medium and mixed with sterilized soil on which the plants were raised. The resulting plants did not show any symptoms of the disease. The roots were normal and had no singns of injury.

(iii) *Pythium graminicolum:* *Pythium* was grown on oat meal and after one week the entire contents of one 250 ml. flask was mixed with sterilized soil in a 9" pot in which two single-eyed setts were planted. All the shoots emerged satisfactorily. Most of them grew without any sign of injury. Only three of them showed curling of the margin and drying of the edges and tips. The plants were taken out at the end of six weeks and the roots examined after washing them free of soil. Affected plants bore damaged roots with a number of red lesions on them. Two apparently healthy plants had stray secondary roots with one or two small lesions. All the others had normal healthy roots. *Pythium* was reisolated from the lesions.

It was felt that the technique was inadequate and that the roots had escaped coming into contact with the pathogen. The fungus was grown on potato dextrose solution and inoculations were carried out in the following two ways:

(a) The setts were planted in sterilized sand and when the shoots had emerged the plants were carefully removed and the roots were washed free of sand without injury. The mycelium of the fungus was lightly beaten up in a mortar with sterile water to get a thick suspension of aggregates of mycelium without dividing the hyphae into too small pieces. The roots were dipped in the suspension and the plants immediately set in sterilized soil and copiously watered.

(b) The second technique was kinglly suggested by Mr. T. S. Ramakrishnan. Single-eyed setts of Co. 467 were planted in sterlized soil in 4" pots with detachable bottoms. After the plants had grown for about two weeks the pot was inverted, the bottom taken out to expose the root tips and the lower portions of the roots. The culture was applied around the exposed ends of the roots without injuring them, sterilized soil was packed around the roots and the bottom replaced. The pots were righted and watered.

The controls in both cases were similarly treated except for the application of the culture.

After one week 12 out of 20 of the plants treated with mycelial suspension and 11 of those inoculated at the root end showed symptoms of root rot. The leaves stiffened and became erect. They dried along the margin and tip and the margins tended to curl inwards. Affected plants died within a few days. There was no chlorosis. The plants were taken out and the roots were examined. There were a number of red coloured rot lesions all along the roots which had been dipped in culture suspension. In the case of the plants which had only their root ends inoculated the lesions were confined to the tip and the portion of the root behind the tip to a distance of up to two inches. There were no lesions in the proximal parts of the root. However the disease lesions appeared to be progressive resulting in root decay. Reisolations yielded *Pythium*.

The controls in either case were healthy looking. The roots were healthy and normal.

(iv) *Effect of mixed inoculation with nematode and Pythium :* Sterilized root rot soil was filled into 10" pots and mixed with a culture of *Pythium* and a suspension of nematodes. Co. 467 was planted. The majority of the shoots (17 out of 20) that emerged were chlorotic with numerous red streaks on the white leaves. The plants remained severely stunted and dried up in three weeks. Three plants germinated normally but after 15 to 20 days they too turned chlorotic and soon withered. The roots were short and severely pitted and covered with red lesions. In some of the cases the sett roots were almost completely rotted back. There was little branching of the roots and the new roots that were produced also showed symptoms of attack. Both nematode and *Pythium* were present as revealed by direct examination as well as by reisolations. The controls were normal as in the other experiments, and were much taller than the diseased ones.

TABLE I

Results of inoculation of Co. 407.

Pathogen added	Number of plants inoculated	No. showing chlorosis	No. showing withering without chlorosis	No. apparently healthy	No. showing root lesions
I Nematodes	20	9	..	11	10 (pits)
II *Pythium graminicolum*:					
(i) Mixed with soil	20	..	3	17	3 (red lesions)
(ii) Root dip in mycelial suspension	20	..	12	8	14 ,,
(iii) Root tip inoculation	20	..	11	9	12 ,,
(iv) Control of each kind	10	..	..	10	.. ,,
III Mixed inoculum	20	20*	..	..	20 (pits and red lesions)
IV Control	20	..	..	20	..

* All the plants withered sooner or later. Three plants were apparently healthy on emergence but turned chlorotic after 15 to 20 days and withered.

(v) *Response to iron spray:* The chlorosis resembles the symptoms of iron deficiency (Martin, 1938). Aqueous ferrous sulphate solution (1 in 1000) was sprayed on the leaves of the affected plants which had been raised on root rot soil in pots. Within 48 hours numerous green islands developed on the sprayed leaves. When the spray was repeated more green spots appeared and coalesced with the older ones. The experiment was also carried out with older plants standing in the field. Only the spindle leaves of these plants responded and the older leaves remained unaffected.

(vi) *Cumulative nature of deficiency:* Setts were cut from plants showing typical symptoms and also from healthy plants growing on wetland clay. A microscopic examination of pieces of tissue of the setts was made. Nematodes could not be detected in them. Both were planted in root rot sick soil. The shoots emerging from the former were yellow and turned white; the plants showed severe stunting and the leaves had a number of streaks. On the other hand the shoots emerging from the setts taken from the healthy plants were normal and green. However, after three or four weeks these also produced chlorotic leaves. The chlorosis was of a milder type and the leaves did not have any red streaks. The setts taken from chlorotic plants were planted in non-root rot soil.

The initial leaves were chlorotic, but soon they turned green and developed normally. Evidently in the absence of nematodes the roots were able to supply the deficiency.

(vii) *Susceptibility of* Saccharum *species*: Observations were made on the standing crop in the root rot sick field. The disease was found to be severe on many of the 'officinarum' canes and the thicker hybrid varieties. In general the thinner canes, particularly those in the *S. barberi* and *S. sinense* groups appeared to be less affected. Variants of *Saccharum spontaneum* did not appear to be affected so severely with chlorosis. Many of them appear to be highly susceptible at the stage of germination. Certain variants appear to be suceptible in the adult stage also. This aspect of varietal resistance is under study.

(viii) *Control of germination failure*: A preliminary slide experiment showed that wettable sulphur was lethal to the nematodes. Young spontaneum plants which showed a mild attack, i. e. those where the shoot had emerged but was making poor progress, were dug out soon after the symptom was detected. The roots were washed in running water and severely affected roots pruned back. The sett with the root system was immersed in a suspension of wettable sulphur (one ounce in $2\frac{1}{2}$ gallons of water) for 10 minutes and planted out in sterile soil. The soil was then given one irrigation with sulphur suspension. Control plants received the same treatment but not sulphur. In two weeks sulphur treated plants recovered by putting on fresh growth and thereafter grew normally while the plants which had not received sulphur did not make progress. Similar results were obtained when nonsterile soil was used, but after a time there was a set back and the symptoms returned indicating that the treatment had not completely eliminated the nematodes.

Discussion: Nematodes have been known to be associated with *Pythium* root rot of sugarcane (Muir and Henderson, 1926). They were believed to facilitate the entry of the fungal pathogen by causing injury to the roots. However in later years nematodes were not thought to be important in the occurrence of the disease. Recent reports have again focussed attention on the injury caused to sugarcane roots by nematodes (Birchfield, 1953). Observations recorded here have indicated that nematodes directly cause slight root injury but the attack leads to a severe from of chlorosis. Heavy nematode infection has been known to be associated with a chlorotic condition of sugarcane (Goodey 1933) and other plants (Good 1956).

The 'Mentek' disease of rice in Java was originally believed to be due to potassium deficiency but ultimately found to be associated with the nematode *Radopholus oryzae* (Van der Vecht, 1953). The affected soil in this Institute has an alkaline reaction (pH 8·4). Response of affected plants to foliar applictions of iron has indicated that an accute deficiency develops following nematode attack. Possibly other elements are also in short supply in the affected plant. This is under investigation. The deficiency is of a cumulative character as revealed by the deterioration of plants from setts taken from chlorotic plants and set in root rot sick soil and by the fact that such setts could make recovery and produce normal plants in the absence of the nematodes on root rot free soil. The mechanism by which a state of deficiency is brought about in the plant is not properly understood. The large scale destruction of lateral roots and root hairs may lead to decreased absorption. But this may be expected to lead to general starvation, and interference with the absorption of water should result in wilting and rapid death. This is probably the case in the young plants which remain stunted and wither away within a short period. However in older plants growth does not altogether cease and there are no symptoms of water deficiency as should be expected if starvation is due to root destruction. The possibility remains of the metabolic products of the nematodes or of the plant roots in response to the presence of nematodes either oxidizing the ferrous iron into the nonavailable ferric state or otherwise chelating with the iron and preventing its absorption.

Pythium graminicolum causes severe root rot but without chlorosis of the leaves. The disease under study appears to be an interesting complex clearly separable into two distinct but complementary aetiological entities, one a *Pythium* root rot and the other a malady associated with nematode injury of the roots and interference with the ferrous iron metabolism of the host. When the two occur together a synergistic effect is apparent and the disease assumes severe proportions. *Pythium* does not appear to be capable of appreciable growth through the soil even when the latter is sterilized. Thus when the fungal culture was mixed with sterilized soil in which setts were planted disease developed only on a few plants as compared to a larger number of plants being affected following direct application of the pathogen to the roots. However when nematodes were present in the soil many of the roots were attacked and yielded *Pythium* on reisolation. Evidently the nematodes help in disseminating *Pythium* through the soil. On account of the attraction

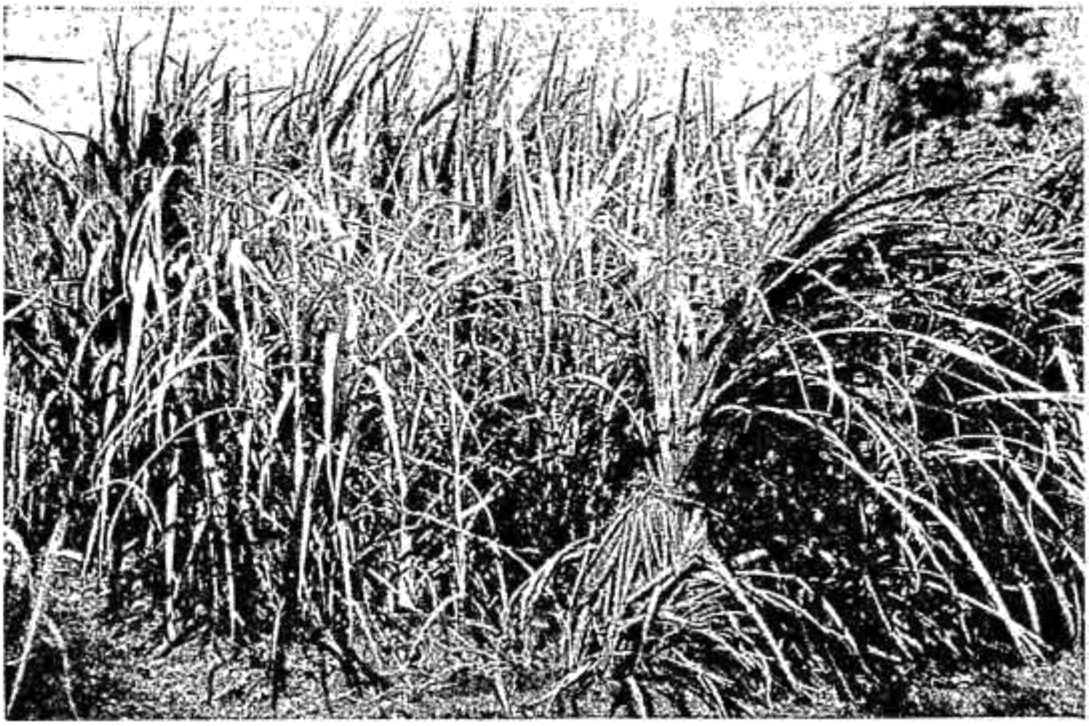

Fig. 1

Sugarcane plants showing Chlorosis

exercised by the roots the worms tend to congregate in the rhizosphere and in the process carry the fungus into this zone of intense activity. Other unpublished studies have in addition revealed that *Pythium* by itself is preferentially stimulated in the rhizosphere of sugarcane. Nematodes appear to play a somewhat different role in the causation of *Pythium* root rot than was assumed by earlier workers (Muir and Henderson, 1926). They seem to be important in building up a pathogenic population in the immediate neighbourhood of the roots and thus help in producing a very severe root rot.

For the purpose of saving plants in pots, treating the roots and drenching the soil around the roots with wettable sulphur has been used with a measure of success. This is particularly welcome as fumigants like chloropicrin, Methyl bromide or Ethylene dibromide cannot be used on living plants. Sulphur appears to be a useful nematicide. Incidentally it is possible that sulphur reduces the alkalinity of the soil by getting oxidized into sulphuric acid and this may make iron available for absorption by the plant and thus mitigate the effects of the disease.

Summary: 1. A disease of sugarcane has been observed characterized by striking chlorosis of the leaves. There may be failure of germination due to rotting of root eyes and bud. Young plants are extremely chlorotic and may soon wither away. Leaves on older plants may turn white but the plants do not die. The root system is decayed.

2. The disease is not confined to any particular species of *Saccharum* but occurs on different species.

3. Nematodes which resemble *Radopholus similis* and the fungus *Pythium graminicolum* are found associated in the diseased roots.

4. When the plants were raised on sterilized soil infested with nematodes severe chlorosis developed.

5. When the plants were inoculated with *Pythium* alone a typical root rot developed but not accompanied by chlorosis.

6. When the plants were raised on soil infested with both nematodes and *Pythium* the symptoms were very severe, with chlorosis of leaves and stunting of plants soon followed by withering of the plants. There was severe root rot.

7. Chlorosis affected plants responded to foliar sprays with ferrous sulphate solution.

8. Young plants of spontaneum showing symptoms of infection, tended to recover when treated with wettable sulphur.

9. It is concluded that the disease is a complex involving two pathogens, nematodes and *Pythium*, the former associated with reduced absorption of iron, and that the coincidence of the two organisms results in synergistic severity of the disease.

Acknowledgment: I am grateful to Mr. N. L. Dutt, Director of this Institute for his sustained interest and encouragement and for critically going through the manuscript and to Mr. R. R. Panje, Botanist, for facilities to make certain of the observations and for useful suggestions. The help of Mr. G. Narasimha Rao with the Baermann Technique is also gratefully acknowledged.

REFERENCES

Birchfield, W. 1953 A parasitic nematode found on deteriorating roots of sugarcane. *Plant Dis. Reptr.* **37** : 38.

Good, J. M. 1956 Plant parasitic nematodes of Georgia. *Ga. agric. Exp. Sta. Univ. Ga. Coll. Agric. Mim. Ser. N. S.* **26** : 1—14.

Goodey, T. 1933 *Plant parasitic nematodes and the diseases they cause.* London.

Martin, J. P. 1938 *Sugarcane diseases in Hawaii.* Honolulu.

Middleton, J. T. 1943 The taxonomy, host rage and geographic distribution of the genus *Pythium*. *Mem. Torr. bot. Cl.* **20** : 1—171.

Muir, F. and Henderson, Gertrude. 1926 Nematodes in connection with sugarcane root rot in the Hawaiian islands. *Hawaii. Planters' Rec.* **30** : 242—245.

Van der Vecht, J. 1953 The problem of the Mentek disease of rice in Java. *Land-bauw*, **25** : 45—130. (Abs. in *Rev. appl. Mycol.* **33** : 558. 1954).