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Relationship between damages of stem, branch and roots, and the pepper vine decline syndrome in Johore

(Hubungan antara kerosakan batang, dahan dan akar dengan sindrom rosot lada di Johor)

A. Mohd. Anuar* and C. F. Loh*

Key words : *Piper nigrum*, vine decline, role of *Lophobaris piperis*, role of root damages

Abstrak

Dalam satu bancian di Stesen Penyelidikan MARDI Kluang, hasil beberapa pokok lada yang kurang subur telah didapati merosot. Kajian menunjukkan bahawa kerosakan buku batang memanjat yang disebabkan oleh kumbang belalai lada, *Lophobaris piperis,* serta kerosakan pada batang bawah tanah dan akar, berkait rapat dengan kejadian daun gugur dan kemerosotan pertumbuhan pokok lada. Jumlah dahan sisi dan sagaran buah juga ketara dipengaruhi secara korelasi negatif oleh jumlah serangan kumbang pada batang memanjat. Begitu juga kerosakan pada akar dan batang bawah tanah turut memberi kesan yang ketara pada hasil dan keadaan pokok. *Fusarium* sp. dan *Pythium* sp. ialah dua pathogen kulat yang sering terdapat pada semua pokok lada yang dibanci, tanpa mengira keadaan dan tahap kecergasan pokok berkenaan. Namun begitu, peranan pathogen berkenaan terhadap gejala kemerosotan pokok mungkin kurang penting.

Abstract

In a survey carried out in MARDI Research Station, Kluang, pepper yield from less-vigorous vines were found to decrease. Damage to the nodes of climbing stems by the pepper weevil, *Lophobaris piperis,* as well as damages to the roots and underground stems were shown to be closely associated with the occurrence of leaf loss and vine decline. The number of lateral branches and fruit spikes were also significantly but negatively correlated with the amount of weevil's damage to the climbing stems. Similarly, root and underground stem damage also affected vine conditions and yield significantly. *Fusarium* sp. and *Pythium* sp. were two fungi pathogens consistently found on all the pepper vines sampled, regardless of their state of vigour and condition. However, the role of these fungi in the decline syndrome was probably secondary.

Introduction

The pepper weevil, *Lophobaris piperis* Mrshl., is a major pest of pepper in Johore. The larvae of the weevil damage vines by boring into the node and internode regions of the lateral branches and climbing stems. This activity disrupts the vascular system and causes the laterals to die or snap off at the node (Kueh 1979; Mohd. Anuar and Loh 1987). Such activities on climbing stems also lead to vine deterioration, especially with recurrent attack (Vecht 1940). Adult weevils feed on the flowers and pericarp of the pepper berries, causing them to fall off

*Division of Horticulture, MARDI, P.O. Box 525, 86009 Kluang, Malaysia
Authors' full names: Mohd. Anuar Abbas and Loh Chow Fong

prematurely or become stunted (Wan 1972; Kueh 1979). In addition, plant pathogens, especially *Fusarium* sp., may enter through the bore points in the climbing stems and cause the larvae tunnels to rot and prevent wound healing (Mohd. Anuar and Loh 1987). Both factors could lead to the decline of the vine (Vecht 1940).

Pepper vines in Johore are also infected by the 'slow wilt', 'slow decline' or 'yellowing' disease (Kueh 1979; Anon. 1981). Symptoms of the disease include foliage yellowing, vine wilt, growth stunting and a general decline in vine vigour. As the vine deteriorates, laterals and leaves are shed but are replaced very slowly. Plant death usually occurs in 1–3 years after the first appearance of the symptoms (Mohd. Anuar and Loh 1987). The decline contributes greatly to the reduction of the economic life span of some pepper vines in Johore, as the disease symptoms may set in as early as the fifth and sixth year of field planting, and by the eighth to tenth year, vines would no longer be economical to maintain. In contrast, vines in well-managed gardens can have an economic life span of 20 years or more as shown by old holdings in India (Nambiar and Sarma 1980).

Various pathogenic and non-pathogenic factors have been associated with the declining vines. Of the non-pathogenic factors, soil deterioration through loss of organic matter and nutrient as well as waterlogging due to poor physical soil conditions can lead to vine decline (Mohd. Anuar and Loh 1987). Pathogenic factors of some importance are root damages by the root-knot nematode, *Meloidogyne incognita* Chitwood and the root burrowing nematode, *Radopholus similis* Thorne. However, their role in the slow decline syndrome is thought to be secondary (Mohd. Anuar and Loh 1987).

Pepper weevil infestations of the vine's stems and branches together with fungi root infections have always been suspected to be important causes of pepper vine decline syndrome in Johore. However, there had been little conclusive empirical evidence to support this suspicion. At the same time, there was no clear knowledge on which of the plant part damage was more serious in influencing vine vigour and yield. The objective of this study was to provide some information on these questions and, at the same time, allow for some avenues of action in controlling the slow decline disease of pepper.

Materials and methods

The study was conducted in a 0.3-ha plot of 6-year-old pepper vines in MARDI Research Station, Kluang, Johore. The vines had been planted on relatively flat land of Rengam series soil, which has a typically low pH and a high sand and clay content. During the study, the vines were at various states of decline although they had been maintained under normal cultural practices.

Weevil damage assessment

For the study, the pepper vines were visually classified into five leaf-loss classes. The classes were

- class 1 = 1–20% leaf loss
- class 2 = 21–40% leaf loss
- class 3 = 41–60% leaf loss
- class 4 = 61–80% leaf loss, and
- class 5 = 81–100% leaf loss

From each class, 25 vines were randomly chosen for destructive sampling. Counts on the number of bore points, both new and old, were taken by examining the nodes of the climbing stem and lateral branches of each vine. To record weevil's damage on lateral branches, a simplification of the count procedure was made by noting the number of laterals present and taking a subsample of 20 randomly chosen branches. Damage counts were made on the subsampled branches. Damage records were then converted to percentage of bored nodes for climbing stems and for laterals. All figures were subsequently reduced to percentages of cumulative node damage for each vine by using the following formula:

Cumulative node damage (%) = $[A + (B/C \times D)] / [E + (F/C \times D)] \times 100\%$ where

- A = number of bored nodes on climbing stems,
- B = number of bored nodes on laterals subsample,
- C = number of laterals sampled,
- D = total number of laterals present,
- E = total number of nodes on climbing stems, and
- F = number of nodes on laterals sampled.

Mean damage values were then calculated for climbing stems, lateral branches and cumulative damage per vine under each class.

The number of flowers and fruit spikes of the sampled vines were also recorded. For this purpose, counts were made from 12 vines selected at random from within each class. All data were analysed using ANOVA followed by DMRT, where applicable. Correlation tests between the variables recorded and regression analyses between damages and yield were also carried out.

Root and underground stem damage assessment

Vines were carefully uprooted and the conditions and damages of the underground stems and roots were visually examined. The vines used were those sampled previously for the weevil damage count. Damage assessment was carried out by classifying the roots and underground stem into three class damage values. The classification was similar for both roots and underground stem. The classes were

- class 1 = healthy roots or underground stem,
- class 2 = moderately (< half) damaged roots or underground stem, and
- class 3 = extensively damaged roots or underground stem.

The state of the vine's roots and underground stem was determined by noting the bark colour, the colour and texture of the cortical tissues when the bark was scraped, and the presence and extent of necrosis and rot. The condition of vine's vascular bundles and the expanse of root mass were also noted. Healthy stems and roots have light grey bark with white or light yellow cortical tissues and normal vascular bundles. Damaged roots have dark bark, grey to dark brown internal tissue, necrotic in some parts and becoming soft and soggy in the advanced disease state (Mohd. Anuar and Loh 1987).

Percentages of healthy roots and underground stems were estimated and classified according to the above class value. To estimate damage severity of root and underground stems for vines within each leaf-loss class, root and stem damage values were reduced to an index of damage. This was done by using the formula adapted from Carlson and Witt (1977) in which, Damage index = $(Sfx)/N$ where S = sum of product, f = number of vines in each class damage value, x = class value, and N = the total number of vines sampled.

Isolations of possible pathogens from roots and underground stems of all sampled vines were also made. Feeder roots from the vines were taken and cultured on PV (pimaracin-vancomycin) media as well as on potato dextrose agar (PDA). All isolates were identified. The number of vines infected by a particular pathogen was also recorded. Damage index data collected were analysed by using the Chi-Square test.

Results

Climbing stem and laterals damage assessment

Analyses of the data collected from these observations showed that there were significant differences ($p = 0.05$) between vines in different leaf-loss classes as shown by counts of node damage on climbing stems, lateral branches, cumulative node damage, number of laterals and fruit spikes (*Table 1*). Vines from class 1, the healthiest among the classes compared, had significantly less ($p = 0.05$) cumulative

Table 1. Severity of damage by the larvae of the pepper weevil, *Lophobaris piperis* and pathogenic fungi on pepper vines at various leaf-loss class values

Class value	Amount of leaf-loss (%)	Mean node damage (%)*			Mean number of lateral branches	Mean number of fruit spikes (n = 12)
		Climbing	Lateral branches subsample	Cumulative		
1	1–20	11.76a	12.98a	12.77a	216.5e	1 895.4e
2	21–40	12.24a	20.53c	18.57b	161.2d	1 385.3d
3	41–60	18.61b	20.06c	19.49b	114.9c	837.8c
4	61–80	18.48b	18.93bc	18.32b	71.1b	468.3b
5	81–100	25.01c	14.87ab	21.82b	26.63a	155.7a

* Means in each column with the same letter are not significantly different at $p = 0.05$ according to DMRT

Table 2. Relationship among vine damage parameters and yield of declining pepper vines *(Piper nigrum)* in Johore

Variables	Amount of bored nodes			Underground stem damage	Root damage	Number of laterals	Number of fruit and flower spikes
	Climbing stem	Lateral branch	Cumulative				
Amount bored nodes on climbing stems	1.000						
Amount bored nodes on lateral branches	–0.76 ns	1.000					
Cumulative damaged nodes	–0.791 ns	0.440 ns	1.000				
Underground stem damage	0.921*	–0.319 ns	0.706 ns	1.000			
Root damage	0.941*	–0.276 ns	0.726 ns	0.995***	1.000		
Number of laterals	-0.942*	–0.149 ns	–0.900*	–0.860 ns	–0.897*	1.000	
Number of spikes	-0.936*	–0.196 ns	–0.893*	–0.822 ns	–0.865*	0.996*	1.000

mean node damage and lateral branch damage than the other classes, except class 5 in the case of lateral branch damage. There were no significant differences in cumulative mean node damage of vines in class 2 to 5. However, damages to the climbing stems increased through the leaf-loss classes with significant ($p = 0.05$) differences between class 2 and 3 and between class 4 and 5, but not between class 1 and 2.

There were also significant differences ($p = 0.05$) in the mean number of lateral branches and fruit spikes between vines in the different classes. Class 1 vines had significantly more lateral branches and hence the most number of fruit spikes ($p = 0.05$). At the same time, significant differences ($p = 0.05$) were also recorded between vines in classes 2, 3, 4 and 5 with regard to the two parameters measured above. Class 5 vines, with the highest amount of leaf loss, had the least amount of laterals and subsequently the least amount of fruit spikes (*Table 1*).

The parameters measured were investigated for their relationship with each other. The number of laterals and fruit

Table 3. Frequency of infections and severity of damage on roots and underground stem of declining vines of various leaf-loss classes

Leaf-loss class value	Vines infected by pathogen (%)		Vines at each root[1]/underground stem[2] damage class (%)			Damage index of root/ underground stem
	Pythium sp.	*Fusarium* sp.	1	2	3	
1	75.0	100.0	100.0/100.0	0/0	0/0	1.00/1.00
2	68.0	100.0	100.0/100.0	0/0	0/0	1.00/1.00
3	42.9	100.0	88.0/ 84.0	12.0/ 16.0	0/0	1.12/1.12
4	40.0	100.0	72.0/ 72.0	28.0/ 28.0	0/0	1.28/1.28
5	65.2	100.0	44.0/ 32.0	44.0/ 48.0	12.0/ 20.0	1.68/1.88

[1] Chi-Square = 44.09, df = 8
[2] Chi-Square = 52.60, df = 8

spikes of a vine were found to be significantly (p = 0.05) but negatively correlated with the amount of node damage to the climbing stems (r = –0.942 and – 0.936 respectively), but not significantly correlated to the amount of damage to the laterals (*Table 2*). However, the number of laterals and fruit spikes present were found to be significantly (p = 0.05) but negatively correlated (r = –0.900 and –0.893 respectively) to the amount of cumulative node damage on the vine. As fruit spikes are borne on lateral branches, the number of laterals retained thus has a significantly (p = 0.05) positive effect (r = 0.996) on the number of fruit spikes present (*Table 2*).

Root and underground stem damage assessment

There were significant differences (p = 0.05) between the leaf-loss classes in terms of the conditions of their roots and underground stem (*Table 3*). There was a tendency for root and underground stem damage to increase through the leaf-loss classes. Vines of class 1 leaf-loss value had no root damage, while vines in class 5 had the highest frequency of partially and severely damaged roots or underground stem among the leaf-loss classes. However, root damage severity, as shown by the damage index, was still within class 1 and 2 for all leaf-loss classes. The severest damage was shown by class 5 vines with a value of 1.68 and 1.88 for roots and underground stem, respectively.

All classes of vines showed the presence of varying amounts of plant pathogenic fungi. Isolations made from vine's feeder roots and underground stem revealed the presence of *Pythium* sp. and *Fusarium* sp. The frequency of isolation showed no particular trend, i.e., no increase over the classes. *Fusarium* sp. was isolated from all vines in all five leaf-loss classes while isolation of *Pythium* sp. was 40.0–75.0% of the vines sampled.

Correlation analyses between the above ground parameters and the underground parts showed some significant relationships. Underground stem and root conditions were significantly (p = 0.05) and positively correlated (r = 0.995) to each other. The number of laterals and fruit spikes were significantly (p = 0.05) but negatively correlated (r = –0.897 and –0.865 respectively) to root damage but not to underground stem damage. The amount of node damage on the vine's climbing stem was also shown to be significantly and positively correlated to root (r = 0.941) and underground stem (r = 0.921) damage. On the other hand, laterals and cumulative node damage were found to be uncorrelated to the damage of the underground parts (*Table 2*).

Discussion

The progress of the pepper vine decline syndrome in Johore can be seen from the gradual loss of laterals, leaves and yield over time. Vine decline, as shown by this study, was found to be significantly related to the amount of damage to the climbing stems, and the severity of damage to the roots and underground stem. As such, the healthiest vines were those showing the least amount of node damage to the climbing stems, had the healthiest underground stem and roots and, consequently, the most number of laterals, leaves and fruit spikes. As damage became more severe to these parts, more and more laterals and leaves were lost. However, damage to the laterals need not necessarily mean loss of fruit spikes as lateral may not abscise in spite of weevil's damage. This was shown by the non-significant nature of the relationship between the amount of node damage on the laterals, and also the number of laterals and fruit spikes ($r = 0.149$ and 0.196 respectively). Lateral abscission is related to lateral size, with bigger laterals having a better chance of staying green and being retained on the vine. Thus, there was no particular trend in the lateral node damage through the classes.

It is difficult to apportioned precisely just how much loss of leaves and laterals were due to weevil damage, and how much of it was due to root damages, as both involved the translocation system. Nevertheless, it is important to prevent damage to the climbing stem and to maintain healthy underground stem and roots. Weevil damage to the climbing stems significantly affected yield as damaged nodes seldom heal, while tunnels made by larvae caused disruptions in the vine's translocation system. As the amount of damaged nodes on climbing stems accumulates over time, vines begin to lose their vigour and the decline syndrome sets in. It is interesting to note that there were significant correlations between the amount of node damage to the climbing stems and the damage status of the vine's roots and underground stem ($r = 0.941$ and 0.921 respectively). This might just be a spurious relationship. However, there was a possibility that weevils had a tendency to attack vines weakened by root problems. Wan (1972) and Kueh (1979) have found that in well managed gardens, vines were not seriously attacked by this insect. Thus, to maintain damage-free climbing stems, judiciously employed prophylactic spraying and drencing with suitable insecticide and fungicide, respectively may be necessary from time to time.

Fungus pathogens, *Pythium* sp. and especially *Fusarium* sp. were found to be present on vines in all leaf-loss classes, regardless of the plant conditions. The presence of these pathogens need not necessarily result in root or underground stem deterioration as shown by the isolations of *Pythium* sp. and *Fusarium* sp. from 75.0% and 100.0% of class 1 vines respectively. In contrast, *Pythium* sp. was isolated from only 40.0% of the more poorer looking class 4 vines which, logically, should yield a higher isolation count. Thus, root damage may be possibly caused by other factors, both pathogenic and non-pathogenic, with these fungi being present only as secondary pathogens on localized infection. However, this was not conclusive. In this study, root or underground damage severity was around the 1.00 unit of the Damage Index, i.e., moderate damage, whereas leaf loss for the same group of vines ranged from normal leaf drop to full foliage loss. Thus, moderate root injuries might significantly affect vine vigour and cause lateral and yield losses. Both *Pythium* sp. and *Fusarium* sp. are predominant in areas where slow wilt is serious (Mohd. Anuar and Loh 1987). *Fusarium* sp. does not harm healthy and vigorously growing vines but would probably only assume parasitic status on weakened plants (Anon. 1983). On the other hand, damage by *Pythium* sp. on vine's feeder roots has been suspected to be a dominant factor in the slow-wilt syndrome (Anon. 1984). Thus, root injuries need to be prevented by good cultural practices, such as avoiding waterlogging, fertilizer and herbicide root scorch, and deep circle weeding (Mohd. Anuar and Loh 1987).

Rough yield estimates can be made visually by determining the amount of foliage present, hence estimating lateral and fruit spike numbers. This is useful for a field census or scouting. Foliage estimation also allows for a rough determination of pepper climbing stem condition. However, for a more accurate vine damage assessment, a closer scrutiny would be necessary. Regression analyses (equation not presented here), between spike number against climbing stem damage and against cumulative node damage, showed a significant negative linear relationship ($p = 0.05$; $r = -0.936$ and -0.893 respectively). At the same time, the analyses also revealed large residual values in both instances, suggesting that the relationships were not strictly linear and that other power curves might fit the data better. Thus, only for a very rough yield estimate, an assumption of a linear relationship between climbing stem node damage and spike number may be adequate.

Conclusion

The study showed that plant decline or slow wilt disease among pepper vines in Johore is the result of a combination of factors. Pepper weevil's damage to the climbing stem of the vine is probably one of the more important contributory factors leading to lateral and leaf losses. At the same time, leaf loss is also the result of damage to the vine's underground system brought about by many causes, with the pathogens *Pythium* sp. and *Fusarium* sp. playing more of an opportunistic role in the slow-wilt syndrome. Hence, these factors need to be controlled to prolong vine life and productivity.

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References

Anon. (1981). Laporan Tahunan 1981, Cawangan Rempah dan Minuman, MARDI, Kluang, p. 14–6 (mimeo.)

____ (1983). Laporan Tahunan 1983, Cawangan Rempah dan Minuman, MARDI, Kluang, p. 18–9 (mimeo.)

____ (1984). Annual Report 1984, Miscellaneous Crops Research Division, MARDI, Serdang, p. 118 (mimeo.)

Carlson, E. C. and Witt, R. L. (1977). Insecticides for *Frankliniella occidentalis* and *Lygus hesperus* on safflower plant. *J. Econ. Ent. 70*: 460–2

Kueh, T. K. (1979). *Pests, diseases and disorders of black pepper in Sarawak* 68 pp. Sarawak: Dept. of Agriculture

Mohd. Anuar, A. and Loh, C. F. (1987). Pests and diseases of pepper, *Piper nigrum* L. in Johore. *MARDI Report No. 118* Serdang: MARDI

Nambiar, K. K. N. and Sarma, Y. R. (1980). Some aspects of epidemiology of foot rot of black pepper. *Proc. of workshop on Phytophthora diseases of tropical cultivated plants* 19–23 Sept. 1980, Kasaragod, India, p. 225–32. Kasaragod: CPCRI

Vecht, J. van der (1940). De kleine peppers-nuitkever (*Lophobaris piperis* Mrshl.), *Meded Inst. Planterz Buitenzorg 97*: 1–44. Cited by Kalshoven, L. G. E. (1981) In *Pests of crops in Indonesia* p. 510–5. Jakarta: P. T. Ikhtiar Baru - Van Hoeve

Wan, M. T. K. (1972). *Pest control* p. 35–41. Sarawak: Borneo Literature Bureau