J. Rubb. Res., **4**(4), 255–269

In-vitro *Evaluation of Exotic* Hevea *Genotypes for Resistance to* Corynespora cassiicola

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Hevea *leaf fall disease caused by* Corynespora cassiicola *(Berk & Curt) Wei, is one of the major disease constraints to the production of natural rubber* (Hevea brasiliensis) *in South Cameroon. Attempts to control this disease by traditionally recommended practices such as artificial defoliation or aerial spray of fungicides have not yielded satisfactory results in Cameroon. Production of disease resistant planting material may provide a more efficient disease control strategy. As part of a screening process of a large pool of* Hevea *genetic resources for disease resistance, we evaluated* in-vitro, *28 genotypes collected from Amazonia. These genotypes were tested for resistance to two isolates of* C. cassiicola *(NK01 and NK02), obtained from different clones showing different levels of susceptibility to the disease in the same field in South Cameroon. The two isolates had similar growth characteristics on culture media. However, isolate NK01 grew faster than NK02 on Potato Dextrose Agar and was also more virulent on the test material. The two isolates may belong to different races. To verify this, further characterisation of the isolates is recommended. Significant differences were observed in the response of the tested* Hevea *genotypes to each of the isolates. Genotypes AC/S/12/22, RO/07/48, and AC/B/19/22 had the highest resistance to both isolates. These could constitute good source material in breeding for disease resistance. Due to their high susceptibility to both isolates, genotypes AC/S/12/02, MT/I/25, MT/C/04/22, MT/C/04/27 and RO/C/08/33 may have to be avoided in any such breeding programme.*

Key words: *Corynespora cassiicola*, disease resistance, *Hevea brasiliensis*, Leaf fall, *in-vitro*, genotypes, breeding, Cameroon

One of the major constraints to natural rubber production world-wide is the high susceptibility of the rubber tree (*Hevea brasiliensis* Muell. Arg) to several diseases. The severity of these diseases varies with climatic conditions and cultural practices in each rubber growing country[1]. Of the common leaf diseases in Cameroon, *Corynespora* Leaf Fall (CLF) due to *Corynespora cassiicola* (Berk & Curt) Wei, is of particular importance, especially in Southern Cameroon characterised by a bimodal rainfall pattern. *Corynespora* leaf fall is a major threat

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to the rubber industry in Cameroon and other African and South East Asian countries[2] The disease is endemic in several rubber growing countries where it causes poor canopy during annual refoliation periods especially if disease outbreak occurs in the later part of refoliation[3] Repeated defoliation frequently results in die-back, especially of young trees[4 5] On mature trees, poor foliage due to leaf disease often causes physiological stress giving rise to stunted girth and a latex yield loss[5] of over 45% In the South province of Cameroon, severe outbreaks of CLF have often been reported with up to 50% – 60% defoliation of PB 260 clones[6] In the same area, total defoliation was observed on clone RRIC 110 during 1998 – 1999, due to *C cassiicola*[7]

Several fungicide recommendations have been proposed for the chemical control of the CLF pathogen[1 3 8] However, to achieve effective control, most of the fungicides required continuous spraying at weekly or even five day intervals for a period of six months[8] Even then, infection was reported to re-occur as soon as spraying was terminated Furthermore different isolates of *C cassiicola* have shown high variability in response to even the most effective fungicide[9], thus making it more difficult to achieve effective control on a large scale This indicates that chemical control does not seem to be economically feasible as pointed out by Jayasinghe and Silva[10]

Artificial defoliation has also been recommended for control of *Corynespora* leaf fall disease[11] especially when it occurs together with *Colletotrichum* and *Oidium* leaf fall diseases as is often the case[8] However, this practice does not seem to yield satisfactory results even when combined with fungicide application and nitrogen fertilisation[12] Similarly, in South Cameroon, both fungicide application and artificial defoliation did not yield any satisfactory results[6]

Considering the high cost of fungicide application (frequent application by aerial spray), their doubtful efficiency in disease control[13] and their likely adverse effects on the environment, alternative strategies of disease control merit greater attention Production of disease resistant planting material would constitute one of such long-term disease control strategies

While there may exist several races of the pathogen, different *Hevea* clones seem to react differently to the fungus[13–17] Evaluation of a large pool of genetic resource would permit identification of disease-resistant material for eventual use in a breeding programme Environmental changes have been shown to modify the susceptibility/resistance responses of some *Hevea* clones to *C cassiicola*[17 19], thus justifying the need to evaluate even known genotypes before introducing them in new agro-ecological environments This study was therefore initiated to evaluate, *in-vitro*, some exotic *Hevea* genotypes for resistance to *C cassiicola* and to compare two isolates of the pathogen obtained from different *Hevea* clones showing different levels of susceptibility to the disease in the same field

MATERIALS AND METHODS

Two isolates of *C cassiicola* (NK01 and NK02) were tested on 28 *Hevea* genotypes drawn from the 1981 Amazonian Germplasm collection of the International Rubber Research and Development Board (IRRDB) The two isolates of the pathogen were respectively obtained from clone RRIC 110 developed by the Rubber Research Institute of Sri Lanka, and accession MT/I/03 from the Amazonian collection, both growing at the experimental field of the National Rubber Research Programme in South Cameroon The test plant

material originated from three Brazilian states of Matto Grosso (MT), Rondonia (RO) and Acre (AC)[20] The experiment was conducted twice in a completely randomised design with three replications Each experimental unit comprised two leaves (six leaflets) from the same plant, freshly harvested at stage B (shiny red – reddish brown) of their development

Pathogen Isolation and Characterisation

From freshly harvested disease leaves, viable lesions were cut, immersed in 95% ethanol for 1 min, rinsed in sterile distilled water, blotted dry on sterile filter paper and incubated on Potato Dextrose Agar (PDA) in the dark for 4 days at 24°C At full growth, the mycelium was sub-cultured repeatedly until pure isolates were obtained The pure isolates were further incubated on PDA in the dark for 6 days and then under light for another 6 days to stimulate sporulation

For characterisation of growth parameters of the pathogen, 5 mm Agar plugs of the mycelium were placed in five replicates on PDA and Potato Carrot Dextrose Agar (PCDA PDA supplemented with carrot extract — 20 gL^{-1}) and incubated in the dark at 28°C for 10 days Colony diameter of the fungus was measured daily until the plate was fully covered with mycelium Growth rate was computed as the daily increase in colony diameter (mm day^{-1}) The growth pattern of the fungus and the sporulation intensity at full growth (conidia counts) on the two media were also recorded Counting of conidia was done with a hemacytometer

Inoculum Preparation

The fungus was grown on PDA in the dark for 6 days and then under light for another 6 days to stimulate sporulation A conidia suspension was then obtained by brushing the conidia with a feather and collecting them in 2 mL sterile distilled water The finial inoculum was obtained by diluting the conidia in sterile distilled water to a concentration of 5×10^4 conidia mL^{-1}

Leaf Inoculation and Evaluation of Infection

Six stage B leaflets from two leaves of the same plant were freshly harvested in dry weather and placed on petri dish lids (three leaflets on each lid) in a plastic trough The leaflets were kept in contact with distilled water at the bottom of the trough by means of a thread of hydrophilic cotton drooping from the leaf petiole The leaflets were inoculated by placing three 10 µl droplets of conidia suspension (5×10^4 conidia mL^{-1}) of each isolate, one on either side of the midrib, on the adaxial surface of the leaflet The troughs were covered with plane glass and an air-tight seal ensured using vaseline. The set up was incubated under light at room temperature for 7 days with daily observation for development of disease symptoms For each isolate, the mean number of lesions per leaflet in each experimental unit (six leaflets) was computed and expressed as Disease Severity Index (DSI)

DSI = (Number of lesions developed/number of inoculated spots) × 100%

Disease incubation period was also recorded as the time interval between inoculation and the first appearance of lesions

Statistical Analyses

All parameters were statistically analysed for Analysis of Variance using STATITCF Means were compared and ranked according

to Neuman Keuls at 5% level of significance. The mean values for the two experiments were considered.

RESULTS

Growth and Sporulation of the Pathogen

Both isolates of the pathogen followed the same growth pattern on both culture media. The mycelium grew uniformly in all directions and was systematically darkened from the centre of the plate towards the periphery. The darkened mycelium was characterised by segmented conidia while in the white zone conidiophores had unsegmented swollen tips. The average growth rate of the isolates calculated over seven days (*Table 1*) showed that on PDA, isolate NK01 had a significantly higher growth rate than NK02 but there was no significant difference in growth rate on PCDA. The growth rate of NK01 was statistically the same on both media whereas NK02 grew significantly faster on PCDA than on PDA. Sporulation was measured as the number of conidia in 1 mL of spore suspension obtained from a total extraction of 2 mL per plate (*Table 1*). Spore counts were analysed both on the numeric and the logarithmic scales. On both scales there were no significant differences between the two isolates in their sporulation intensity. There was also no significant effect of culture medium on sporulation.

Susceptibility of *Hevea* Genotypes to *C. cassiicola*

Susceptibility of the genotypes to both isolates of the pathogen was determined from the severity of disease development on the leaves within one week, as well as from the disease incubation period (number of days taken for first disease symptoms to appear). Disease severity index recorded after 3, 5 and 7 days of incubation and disease incubation period revealed significant differences between the genotypes. The most severe infection by NK01 was recorded on genotypes AC/S/12/02, MT/C/04/27, MT/C/04/22 and MT/I/25 while genotypes AC/S/12/22, MT/C/02/13 and RO/07/48 had the lowest disease development (*Table 2*). The shortest incubation period of isolate

TABLE 1. GROWTH RATE AND SPORULATION OF *CORYNESPORA CASSIICOLA* ISOLATES ON PDA AND PCDA

Treatment	Growth Rate (mm day^{-1})	Sporulation (x 10^4 conidia mL^{-1})
NK01 on PDA	9.6a	2.45
NK01 on PCDA	9.5a	1.98
NK02 on PDA	9.1b	1.59
NK02 on PCDA	9.7a	1.92

Within the column, numbers followed by different letters are significantly different at 95% confidence level.

TABLE 2. RESPONSE OF *HEVEA* GENOTYPES TO *CORYNESPORA CASSIICOLA* ISOLATE NK01

Accession code	DSI: Day 3	DSI: Day 5	DSI: Day 7	Incubation period
MT/C/04/22	64.7 abc	100.0 a	100.0 a	2.0 bcd
AC/S/12/02	89.0 a	98.2 ab	98.2 ab	1.0 d
MT/I/25	57.4 abcd	98.2 ab	98.2 ab	1.0 d
MT/C/04/27	81.3 ab	90.7 abc	90.7 abd	1.0 d
MT/C/02/14	14.8 def	87.0 abcd	90.7 abc	2.3 bcd
MT/C/02/17	27.8 cdef	85.2 abcd	90.7 abc	2.7 bc
AC/S/13/04	0.0 f	83.3 abcd	92.6 abc	4.3 b
AC/X/21/10	40.7 cdef	81.5 abcd	81.5 abcd	2.0 bcd
RO/PB/02/11	9.3 ef	81.5 abcd	100.0 a	2.7 bc
RO/I/11/03	22.2 cdef	79.6 abcde	79.6 abcd	1.7 bcd
MT/C/04/11	1.9 f	77.8 abcde	77.8 abcd	4.0 b
RO/C/08/33	57.3 abcd	75.9 abcde	75.9 abcd	1.0 d
AC/S/12/35	37.0 cdef	75.9 abcde	75.9 abcd	1.0 d
MT/C/02/38	24.1 cdef	74.1 abcde	74.1 abcd	2.0 bcd
AC/S/13/06	27.8 cdef	72.2 abcde	72.2 abcd	2.0 bcd
MT/C/01/01	18.5 def	72.2 abcde	81.5 abcd	2.3 bcd
MT/C/01/16	50.0 bcde	70.4 abcde	70.4 abcd	3.0 b
AC/S/12/26	46.3 cde	70.4 abcde	70.4 abcd	2.3 bcd
AC/B/19/22	29.6 cdef	61.1 abcde	61.1 abcd	3.0 b
RO/C/09/39	14.8 def	59.3 abcde	75.9 abcd	3.0 b
AC/I/05	42.6 cdef	57.4 abcde	57.4 abcd	1.3 cd
RO/J/06/03	29.6 cdef	55.6 abcde	66.7 abcd	2.0 bcd
MT/C/05/20	25.9 cdef	53.7 bcde	53.7 abcd	2.0 bcd
MT/IT/18/04	29.6 cdef	50.0 cde	51.9 abcd	2.0 bcd
RO/JP/03/35	24.1 cdef	50.0 cde	57.4 abcd	2.0 bcd
RO/07/48	0.0 f	44.4 de	51.9 bcd	5.0 a
MT/C/02/13	14.8 def	37.0 de	37.0 d	3.0 b
AC/S/12/22	0.0 f	11.1 f	40.7 d	5.0 a

In any column, values with no letter in common are significantly different at 95% confidence level.

NK01 was recorded on genotypes AC/S/12/02, MT/C/04/27, MT/I/25, RO/C/08/33 and AC/S/12/35 where the first disease symptoms appeared within one day of incubation. Incubation period was longest on genotypes AC/S/12/22, RO/07/48, AC/S/13/04 and MT/C/04/11 where it took at least four days for disease symptoms to appear (*Table 2*). The trend of disease evolution on some of the genotypes due to NK01 is given in *Figure 1*.

The highest levels of infection by isolate NK02 were recorded on genotypes AC/S/12/02, MT/C/04/22, MT/I/25 and RO/PB/02/11. The lowest infection occurred on AC/S/12/22, MT/C/02/13, AC/I/05 and AC/S/12/35 (*Table 3*). As with NK01, the longest incubation period of NK02 was recorded on genotypes AC/S/12/22, RO/07/48, AC/S/13/04 and MT/C/04/11. Similarly the shortest incubation period was observed on genotypes AC/S/12/02, MT/C/04/27, MT/I/25, RO/C/08/33 and RO/I/11/03 (*Table 3*). The trend of disease evolution on some of the genotypes due to NK02 is given in *Figure 2*.

To evaluate the relative aggressiveness of the isolates on individual genotypes, the average disease development over seven days was compared. On the genotypes shown in *Figure 3*, significant differences were observed, NK01 always producing more severe attack than NK02. A comparison of the incubation period on individual genotypes showed no significant differences between the isolates except for AC/X/21/10 on which NK02 had a significantly longer incubation period (3.33 days) than NK01 (2 days). The average disease severity due to isolate NK01 was significantly higher than that due to NK02, with a corresponding significantly shorter incubation period.

DISCUSSION

Growth and Sporulation of the Pathogen

The two isolates of the pathogen showed the same growth pattern on both culture media, suggesting some similarity between the isolates. This similarity was further seen in the degree of sporulation where no significant differences were recorded between the isolates, contrary to an earlier report of high variability in spore morphology and sporulation intensity within *C. cassiicola* populations[21]. Despite this similarity, there seemed to be a physiological difference between the two isolates as seen in the differences in growth rates on the two culture media. PCDA is a nutritionally richer medium than PDA. While medium enrichment was seen to significantly speed up the growth of NK02, there was no such effect on NK01. This difference in response to nutrient medium may indicate an important difference between the isolates. However, the growth parameters studied in this work do not permit a clear determination of the similarity or difference between the isolates. Further physiological and possibly, biochemical characterisation would be recommended.

According to Dhingra and Sinclair[22], a nutritionally rich medium would favour vegetative growth at the expense of sporulation, which is generally an indication of nutritional exhaustion. Despite the accelerated vegetative growth of NK02 on PCDA, sporulation intensity was similar on both media and comparable to that of NK01. This suggests that nutrients were probably sustained to the same level in both media. We conclude that simple PDA is sufficiently suitable to culture *C. cassiicola*.

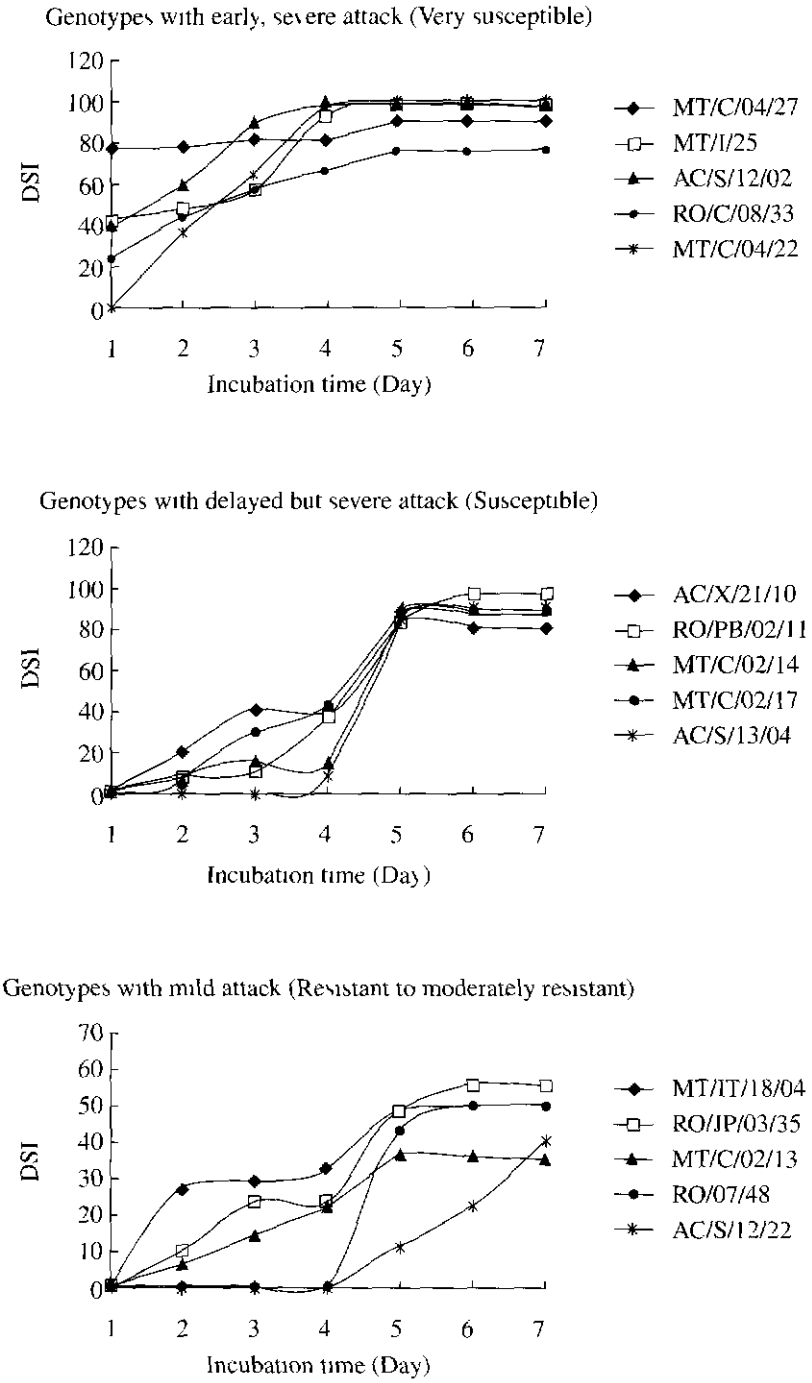


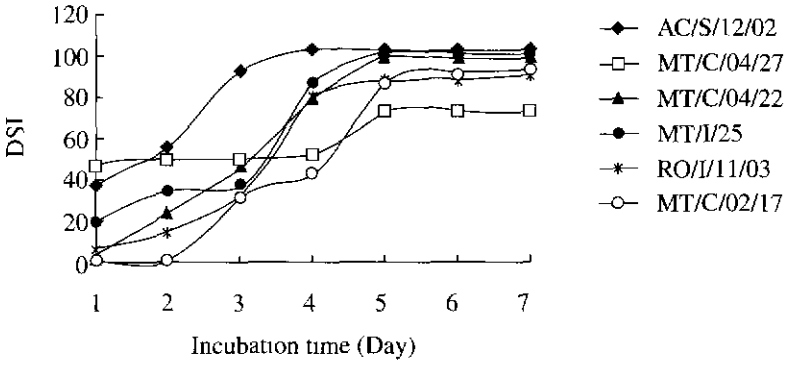
Figure 1. Evolution of Corynespora cassiicola *NK01 on some* Hevea *Genotypes.*

TABLE 3. RESPONSE OF *HEVEA* GENOTYPES TO *CORYNESPORA CASSIICOLA* ISOLATE NK02

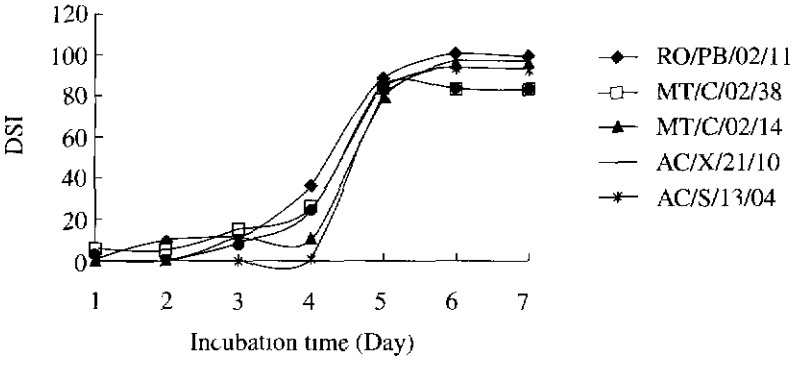
Genotype code	DSI: Day 3	DSI: Day 5	DSI: Day 7	Incubation period
AC/S/12/02	87.0 a	96.3 a	96.3 ab	1.0 f
MT/I/25	35.2 bcd	94.4 a	94.4 abc	1.0 f
MT/C/04/22	42.6 bc	92.6 a	92.6 abc	2.0 cdef
RO/PB/02/11	13.0 bcd	88.9 ab	100.0 a	2.0 cdef
RO/I/11/03	31.5 bcd	83.3 abc	83.3 abcde	1.3 ef
MT/C/02/17	29.6 bcd	83.3 abc	87.0 abcd	3.0 cdef
MT/C/02/38	14.8 bcd	83.3 abc	83.3 abcde	1.7 def
AC/X/21/10	9.3 bcd	83.3 abc	83.3 abcde	3.3 cde
MT/C/02/14	11.1 bcd	79.6 abc	96.3 ab	3.7 bcd
AC/S/13/04	0.0 d	79.6 abc	92.6 abc	5.0 ab
MT/C/01/01	9.3 bcd	70.4 abcd	70.4 abcdef	2.7 cdef
MT/C/04/27	46.3 b	68.5 abcd	68.5 abcdef	1.0 f
AC/S/13/06	9.3 bcd	68.5 abcd	68.5 abcdef	2.3 cdef
RO/C/08/33	18.5 bcd	66.7 abcd	66.7 abcdef	1.3 ef
RO/JP/08/33	22.2 bcd	61.1 abcd	63.0 abcdef	2.0 cdef
MT/C/01/16	42.6 bc	61.0 abcd	61.1 abcdef	3.0 cdef
AC/S/12/26	44.4 bc	59.3 abcd	59.3 abcdef	2.0 cdef
MT/C/04/11	0.0 d	57.4 abcd	57.4 abcdef	4.0 bc
AC/B/19/22	11.1 bcd	51.9 abcd	51.9 abcdef	3.3 cde
MT/C/05/20	7.4 cd	51.9 abcd	51.9 bcdef	3.0 cdef
MT/IT/18/04	27.8 bcd	44.4 bcd	51.9 bcdef	2.0 cdef
AC/S/12/35	7.4 cd	44.4 bcd	44.4 def	2.3 cdef
RO/07/48	1.9 d	44.4 bcd	50.0 bcdef	5.0 ab
RO/C/09/39	13.0 bcd	40.7 cd	48.1 cdef	2.7 cdef
RO/J/06/03	14.8 bcd	38.9 cd	64.8 abcdef	2.0 cdef
AC/I/05	14.8 bcd	33.3 de	33.3 f	2.3 cdef
MT/C/02/13	16.7 bcd	29.6 de	29.6 f	1.7 def
AC/S/12/22	0.0 d	3.7 e	38.9 ef	5.7 a

In any column, values with no letter in common are significantly different at 95% confidence level.

Genotypes with early, severe attack (Very susceptible)



Genotypes with delayed but severe attack (Susceptible)



Genotypes with mild attack (Resistant to moderately resistant)

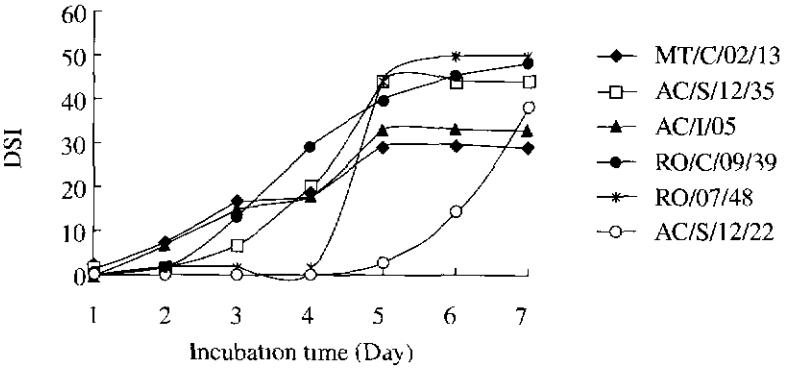
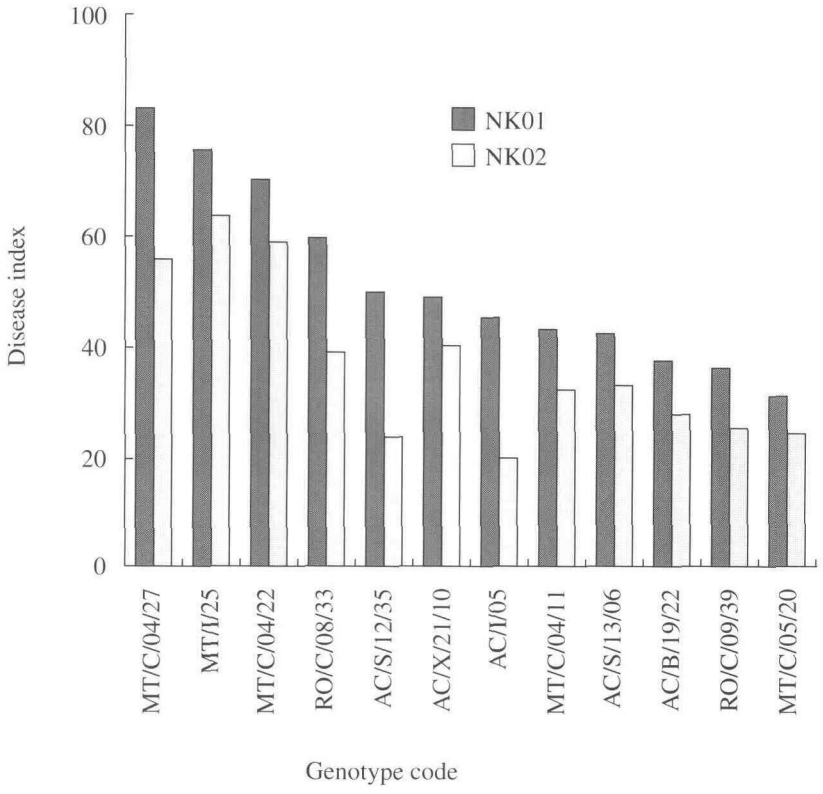


Figure 2 Evolution of Corynespora cassiicola *NK02 on some* Hevea *Genotypes*



For each accession, isolate NK01 produced significantly higher average disease index at 95% confidence level.

Figure 3. Comparative aggressiveness of C. cassiicola *isolates on some Rubber genotypes.*

Susceptibility of *Hevea* Genotypes to *C. cassiicola*

Infection of plant tissue by a pathogen occurs after successful germination of the spore on the surface of the host organ and penetration of the germ tube into the host tissue. The host plant may evade infection by physically preventing spore fixation on its surface or penetration of the germ tube. Even when infection is successful, the host plant can be induced to put up defence reaction that hinder disease development[15,23–25]. Different genotypes of the same species may be expected to present some morphological variations which may affect their specific host-pathogen interactions. The response of a given genotype may also vary among different isolates of a pathogen since the pathogen exerts a number of reactions to counter the defence mechanisms of the host plant[25]. These characteristics of host-pathogen relationships may explain the differences in response of several genotypes of a species to the same pathogen isolate or of the same genotype to

different pathogen isolates as has been revealed in this study. The differences reported in this paper between the isolates and between the genotypes agree with earlier reports that different isolates vary in pathogenic effects, causing varying levels of infection on different clones[15–17,21,26,27]. These differences can be explained by the recent findings by Breton *et al.*[28] that the pathogenicity of *C. cassiicola* may be strongly linked to the production of a toxin (cassiicolin) by the fungus. While different isolates of the pathogen have been shown to produce different amounts of the toxin, different *Hevea* genotypes also respond differently to different concentrations of the toxin, with higher amounts needed to produce symptoms on the more resistant clones[28]. However, some genotypes have shown the same degree of resistance or susceptibility to both isolates. This conforms with observations by Sinulingga *et al.*[14] that five isolates of *C. cassiicola* from *Hevea* clones with different degrees of susceptibility to the pathogen produced the same level of pathogenicity to clone GT1.

According to Hashim and Chew[8], *Hevea* leaves are most vulnerable to *Corynespora* attack during their early stage of development when the leaves are still very tender. Genotypes on which the pathogen has a long incubation period may be expected to show resistance in field conditions since the leaves would harden before the pathogen has time for infection. Breton *et al.*[28] have shown that *C. cassiicola* directly penetrates the rubber leaf epidermis within 12 h after inoculation, irrespective of the resistance or susceptibility characteristics of the clone. This indicates that resistance reactions are stimulated after pathogen penetration. As shown by these authors, pathogenicity of *C. cassiicola* is largely owed to the production of a toxin (cassiicolin) and the amount of the toxin required to produce disease symptoms varies among clones. Disease symptoms become visible as lesions after the collapse of epidermal cells following cassiicolin secretion. While susceptible genotypes may be expected to show disease symptoms within 24 h[24] in response even to low amounts of cassiicolin, visualisation of symptoms in the less susceptible genotypes may be expected to delay for two possible reasons: low amounts of cassiicolin secreted at the beginning of infection may only provoke slow collapse of cells in the neighbourhood of infection points, thus requiring a long time for the collapsing cells to produce a visible lesion; secondly, the toxin secreted at the onset of infection may need to build up until the amount is high enough to produce toxic effects. We therefore postulate that the number of lesions developed and the time it takes for such lesions to develop could give a good indication of the degree of susceptibility or resistance expressed by the plant. Due to this, we propose a susceptibility/resistance rating for the tested genotypes on the basis of the severity of infection (DSI) and the incubation period according to the following scale (*Scheme 1*)

The disease situation at day 5 has been used in this scheme because disease development stabilised on most of the genotypes after 5 days (*Figures 1* and *2*). A classification of the tested material according to this scheme is given in *Table 4*. This classification scheme reveals that genotypes AC/S/12/22, RO/07/48, AC/B/19/22 and to a lesser extent, MT/C/02/13, RO/C/09/39 and MT/C/05/20 had the highest resistance to both isolates of the pathogen. They may therefore constitute good source material in breeding for resistance to *C. cassiicola*. Meanwhile, genotypes AC/S/12/02, MT/I/25, MT/C/04/27, RO/C/08/33, MT/C/04/22 and to a lesser extent, RO/PB/02/11, AC/S/13/06, RO/I/11/03, MT/C/01/01 and MT/C/02/38 seem to be very susceptible and may have to be avoided in such a breeding programme.

Rank	DSI at Day 5	Incubation period	Description
1	< 67	≥ 3 days	Resistant
2	< 67	< 3days	Moderately resistant
3	≥ 67	≥ 3 days	Moderately susceptible
4	≥ 67	< 3 days; DSI at day 3 < 50	Susceptible
5	≥ 67	< 3 days; DSI at day 3 > 50	Very susceptible

Scheme 1

TABLE 4. SUSCEPTIBILITY CLASSIFICATION OF *HEVEA* GENOTYPES TO *C. CASSIICOLA* ISOLATES

Isolate	Susceptibility ranks				
	1	2	3	4	5
NK01	AC/S/12/22 RO/07/48 MT/C/02/13 RO/C/09/39 AC/B/19/22	RO/JP/03/35 MT/IT/18/04 MT/C/05/20 RO/J/06/03 AC/I/05	MT/C/04/11 MT/C/01/16 AC/S/13/04	AC/S/13/06 MT/C/02/38 AC/S/12/35 AC/S/12/26 MT/C/01/01 RO/PB/02/11 MT/C/02/17 AC/X/21/10 MT/C/02/14 RO/I/11/03	RO/C/08/33 MT/C/04/22 MT/C/04/27 MT/I/25 AC/S/12/02
NK02	AC/S/12/22 RO/07/48 MT/C/04/11 AC/B/19/22 MT/C/05/20 MT/C/01/16	AC/I/05 RO/C/09/39 RO/J/06/03 MT/C/02/13 AC/S/12/35 MT/IT/18/04 AC/S/12/26 RO/JP/03/35	AC/S/13/04 MT/C/02/14 AC/X/21/10 MT/C/02/17	MT/C/01/01 AC/S/13/06 MT/C/02/38 RO/PB/02/11 MT/C/04/22 RO/C/08/33 MT/C/04/27 RO/I/11/03 MT/I/25	AC/S/12/02

1 = Resistant; 2 = Moderately resistant; 3 = Moderately susceptible; 4 = Susceptible; 5 = Very susceptible.

The classification scheme in *Table 4* also shows that almost all the genotypes tested had either the same or higher levels of susceptibility to NK01 compared to NK02. Further more, on all genotypes where significant differences in DSI were recorded between the isolates, this was always higher for NK01 than NK02. The average disease severity due to NK01 was also significantly higher than that due to NK02. These differences show that isolate NK01 is more virulent than NK02. Field observations showed that clone RRIC 110 from which NK01 was isolated suffered very severe leaf fall due to *C. cassiicola* with nearly 100% defoliation shortly after the annual physiological refoliation while only mild to moderate attacks were recorded on MT/IT/03 from which NK02 was isolated[7]. This further strengthens the opinion that the two isolates are significantly different from each other despite the morphological similarity between them. Darmono *et al.*[29] also observed that differences in virulence between *C. cassiicola* strains are not associated with groupings of the isolates on basis of molecular and morphological characters. Therefore, although we suggest that the two isolates may possibly be of different races, we cannot conclude, on the basis of the differences in virulence and the morphological similarities observed between them in this study, whether or not they belong to different races. Further characterisation of the isolates and cross infection assays would be recommended.

Date of receipt: May 2001
Date of acceptance: November 2001

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