Chapter 6

GENERAL CONCLUSION

SUMMARY AND CONCLUSION

In this study it was observed that TPD is initiated mainly by two ways either with extreme fluidity of the latex with abnormally low DRC in which the latex flows only from the inner most layer of latex vessels or with very viscous latex with abnormally high DRC and partial dryness of varying degrees with vessels devoid of latex. When dryness was observed in the BO 1 panel, the cracks were seen extended to the opposite panel. Cracks and necrosis started from the tapping panel or from the region near the bud union, which later extended towards the tapping cut. The present study revealed that cracking and bulging of bark increased with the age of trees and with progression in period of tapping. Nearly 40% reduction in total latex volume was observed on trees in the category of less than 50% TPD and nearly 90% reduction was observed when the trees were in the category of more than 75% TPD in panel BO 1. Contrary to total latex volume, an increase in DRC was noted as the TPD intensity increased. Dryness of root system was observed along with necrosis, cracking and bulging (as observed on the trunk) in TPD affected trees. Although the roots originated from a root stock those on the side corresponding to the dry portion of scion showed dryness.

It was observed that the number TPD affected trees increased as the years of tapping progressed at all the locations both in small holdings and in large estates. The percentage of trees in the category of very high TPD intensity (>75%) showed a clear trend of increase from the first year to the last year of tapping at all the locations. The number of TPD trees in the other categories (low, medium and high) did not show such a remarkable trend of increase from BO 1 to BI 1 panels. The scale of increase in TPD was more in older trees than in trees at the initial stages of tapping. Percentage of trees without TPD symptoms was high when the panel was changed but it again decreased a year after such panel change. Reversion of TPD symptoms was observed only at a young age. The evidence for natural transmission of TPD from one tree to the other was observed in the present field studies as number of trees in clusters showed a significant increase with progress of tapping. When the systems for management of TPD were studied, it was found that only 23.8% of the small holders give rest when TPD was observed. When the TPD affected trees were tapped in the upward system of tapping, more than 50 per cent of the trees showed dryness after four months of tapping.

Various factors which were assumed to be the cause of TPD could not conclusively prove its etiology. Occurrence of TPD in adjacent trees in the same line, consistent increase in its incidence with age, similarity in symptoms and anatomical abnormalities to some known diseases, inability to revive the affected trees by tapping rest and the presence of dry rubber trees from the start of exploitation prompted investigation on biotic etiology for TPD. Earlier investigations did not reveal association of fungi, bacteria, virus, MLO or protozoa as biotic agents. Hence, possibility of association of viroid with TPD syndrome was investigated. A protocol was standardized to detect the presence of LMW RNA in leaf, bark and root tissues of rubber trees. LMW RNA was detected from different samples drawn from varying ages of trees from different locations. In most cases the trees in which LMW RNA was detected showed the TPD syndrome. Therefore, it was hypothesized that the presence of LMW RNA has an association with TPD.

Presence of bands in apparently healthy samples indicated that the biotic agent also occurs in symptomless carriers. Majority of healthy trees that earlier found positive for LMW RNA eventually became TPD affected. Hence, this technique can be used as a diagnostic tool for detection of TPD before the symptoms are visible. The amplified LMW RNA showed sequence homology to Potato Spindle Tuber Viroid (PSTVd).

The R-PAGE test of bud grafted plants under transmission studies showed that all the plants tested from the group in which both stock and scion were viroid +ve, maintained the viroid bands. Viroid was observed to be transmitted from viroid +ve stock to viroid –ve scion. Test tapping showed TPD in both group of plants, namely plants budded with scion taken from TPD affected trees as well as those from apparently healthy trees. This shows that root stock also plays a role in the development of TPD. Development of epinasty symptom on tomato plants inoculated with total RNA isolated from TPD affected trees showed that the viroid present in rubber can be transmitted to an indicator host indirectly satisfying the Koch’s postulates. It was reisolated and the LMW RNA band was observed in R-PAGE analysis of reisolated sample. The sequence homology of the RT-PCR product obtained from the inoculated tomato with that of Potato Spindle Tuber Viroid proved its viroid nature.

Although the primary natural host of PSTVd is potato, it is reported that this viroid also infects a variety of other crops. It has a wide host range, infecting 94 species in 31 families (Jeffries, 1998). In Peru, PSTVd has been detected in Avocado (*Persea* *americana*), which is a perennial tree species where infections are often latent unless the tree is co-infected with Avocado Sun blotch Viroid (Querci *et al*., 1995). The spread of the viroid into new cultivars of woody perennials can occur through vegetative propagation as suggested by Bar-Joseph (2003).

The most appropriate method to establish viroid etiology for TPD is artificial transmission of the LMW RNA isolated from rubber to a viroid free healthy rubber plant to express TPD symptoms. However, the varying periods of latency of the viroid (over several years) prior to symptom expression, as observed in this study, poses problems. If viroid etiology is confirmed, bud wood and root stock certification for pathogen-free propagation materials can be adopted for developing TPD free plantations.