UNIVERSITI PUTRA MALAYSIA

DECOMPOSITION OF ROOTS AND NODULES OF *CENTROSEMA PUBESCENS* BENTH.

AZIZAH BT HASHIM

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This thesis is dedicated to my
beloved husband and two sons.

DECOMPOSITION OF ROOTS AND NODULES OF *CENTROSEMA PUBESCENS* BENTH.

by

Azizah Bt Hashim

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ABSTRACT

A modified technique involving incubation of moistened Serdang Series soil samples with segments of radicle and lateral roots as well as portions of nodules of *Centrosema* was used to study release of nitrogen into the soil as well as the biology of decompositon of roots and nodules of this legume. The samples were incubated for various lengths of time under optimum temperature and moisture content. At weeks 0, 1, 2, 4, 8, 12 and 16, the respective soil samples were cleared of undecomposed legume tissues and analysed for total N, ammonium, nitrate, organic carbon and soil pH. At the same time the extracted laterals, radicle and nodule tissues were respectively plated on Czapek-Dox and potato dextrose agar media for isolation and enumeration of the decomposer mycoflora.

The amount of ammonium nitrogen released from the different soil treatments was much higher than the levels of nitrate nitrogen released throughout the experiment. This could probably be due to the slightly acidic nature of the soil media used. The organic carbon content however decreased with increase in incubation time. This ultimately resulted in the narrowing of the C/N ratio to < 10 for all treatments under study. Accumulation of the ammonium nitrogen in the soil apparently resulted in the slight increase in soil pH. Soils amended with nodules varied significantly for all analyses when compared to the other treatments.

The number of decomposer mycoflora isolated from the three tissue types increased with advanced tissue decay. For all the three tissue types used: laterals, radicles and nodules, five fungal genera were isolated frequently throughout the sampling period. The different

genera isolated were *Fusarium*, *Trichoderma*, *Curvularia*, *Gliocladium* and *Penicillium*. Total number of fungal genera and total isolates from decaying nodules were significantly higher when compared to results obtained from either decaying laterals or radicles.

Chapter 1

Introduction

Symbiotic systems of nitrogen fixation have long been recognized to be an important source of nitrogen for agricultural crops. In fact, it has been estimated that of the 10^8 tons of nitrogen fixed annually, the largest portion comes from symbiotic sources, especially from the nodules of leguminous plants growing in natural associations and in agriculture (Donald, 1960). In New Zealand, under favourable conditions, symbiotic fixation has been reported to amount to as much as 683 kg N. $ha^{-1} . y^{-1}$ (Sears *et al.*, 1965) while in Britain, maximum quantities are probably between 340 - 455 kg N. $ha^{-1} . y^{-1}$.

Delwiche in 1970 estimated that alfalfa and other leguminous crops contribute about 350 kg N. $ha^{-1} . y^{-1}$ or approximately 100 times the annual rate of fixation attainable by non-symbiotic organisms in a natural ecosystem. Fixation of nitrogen by other legumes as found out by Spector in 1956 (Nutman, 1965) in terms of kg N per hectare per year are as follows:- clovers, 58 - 795; peas, 35 - 160; peanuts, 100, and pastures with legumes, 13 - 625.

Symbiotically fixed nitrogen can be made available to other plants in several ways. One is through the death of the legume host tissues. During this time, the fixed nitrogen becomes available to other plants through the normal process of mineralization. Secondly, investigations by several workers like Bond and Boyes (1939), Wilson and Burton (1938) and Ludwig and Allison (1940) showed that nitrogen can be excreted into the soil by the living legume roots. However, much experimental work has failed to produce convincing evidence to support the significance of

the above phenomena in agricultural practices. Release of nitrogen through excretion is therefore still debatable.

Symbiotically fixed nitrogen can also be made available to associated non-legume plants by the "direct" nitrogen transfer mechanism (Simpson, 1976). This mechanism was referred to as "underground transfer" by Butler and Bathurst (1956). The "direct" nitrogen transfer involves an exchange of nitrogen between legume roots and grass roots, either simultaneously or after a certain period of decomposition in the soil. Simpson (1976) found that large amounts of nitrogen were transferred to grass plus soil in this manner, i.e. about 100 - 190 kg N. ha^{-1} . y^{-1}.

In terms of soil fertility in tropical regions, inclusion of a legume *(Centrosema pubescens)* with giant stargrass *(Cynodon plectostachyus)* has been found to increase the organic matter, total nitrogen and also nitrifiable nitrogen in the underlying soil when compared to a pasture without any legume (Moore, 1962). In fact, the total nitrogen content under the pasture containing the legume was 285 kg N. ha^{-1} higher than that under the pure grass alone. Inclusion of leguminous species in pasture mixtures has become a very common practice in temperate agriculture. The aim of this practice is to improve the fertility of the soil under the pasture. Simpson (1976) in his studies showed that the average annual inputs of nitrogen by the legumes in mixed swards were high, exceeding 500 kg N. ha^{-1} . y^{-1} for lucerne.

Studies done by several workers including Whitehead (1970) have shown that the nitrogen fixed by legumes increases soil nitrogen more effectively than does either fertilizer nitrogen applied to all-grass swards, or nitrogen gained by non-symbiotic fixation. Normally, when legumes decay, up to half of their nitrogen content becomes available to

plants within a short time. The remainder, however, is released more slowly (Vallis and Jones, 1973). They investigated the possible causes for the slow release of nitrogen from leaves and litter of *Desmodium intortum cv. Greenleaf* in comparison to *Phaseolus atropurpureus cv. Siratro*. The leaves and litter were incubated with soil at 24°C for up to 32 weeks. They discovered that nitrogen mineralization with *Desmodium intortum* was less than with *P. atropurpureus* for both leaves and leaf litter. In fact after 32 weeks, only 3% of the nitrogen in *Desmodium* leaves had been mineralized, compared to 47% for *Siratro*.

Analysis done on both the legume leaves and litter showed that the concentration of polyphenols in *D. intortum* was twice that in *P. atropurpureus*. For instance, total polyphenols for leaves and litter of *D. intortum* amount to 4.5% and 4.5% respectively, while total polyphenols in leaves and litter of *P. artropurpureus* amounts to 2.2% and 2.0% respectively. The high polyphenol (tannic acid) contents of *Desmodium* leaves have been associated with the slow rate of decay of these leaves. It has been suggested that the polyphenol formed complexes with protein which resist microbial decomposition of the leaves, hence the slow rate of decay.

I ENTRY OF NITROGEN INTO THE SOIL

One of the ways in which nitrogen enters the soil is through the decay of the legume root nodules. This usually happens when the carbohydrate supply to the nodule is restricted, resulting in the sloughing off of the nodule tissues (Butler and Bathurst, 1956). Other factors which enhance decomposition of legume root nodules and therefore accelerate nitrogen release into the soil include fruiting, extremes of soil temperature or soil moisture content (Wilson, 1931); defoliation (Wilson, 1942) and pronounced shedding (Strong and Trumble, 1939).

Other routes of nitrogen entry into the soil include excretion of nitrogenous compounds by legume roots and nodules (Butler and Bathurst, 1956) in association with grass pastures; the sloughing-off and decay of legume root tissues; leaching of nitrogenous compounds from legume leaves by rain water, decay of legume leaves and decapitated petioles (Butler and Bathurst, 1956).

Of the routes mentioned above, decomposition of legume root nodules release the most nitrogen to the soil. Butler and Bathurst (1956) estimated that in a pasture containing grass and white clover, the amount of nitrogen released by decaying clover nodules amounted to 83 kg N. ha^{-1}. y^{-1}. Second highest nitrogen release came from decaying root tissues of legume which contributed about 40 kg N. ha^{-1}. y^{-1}.

Excretion of soluble organic compounds such as amino acids, aspartic acid and β-alanine by legume roots and nodules has been difficult to prove both in the laboratory as well as in the field (Russell, 1973). However, Virtanen and Laine (1939) showed the excretion of such compounds by *Rhizobium* - inoculated pea plants under aseptic conditions. The significance of the nitrogenous excretion by the legume has yet to be deter-

mined.

The two other processes which contribute a small amount of nitrogen to the soil are leaching of nitrogenous compounds from legume leaves by rain and also through the decay of leaves and decapitated petioles of legumes. Most of the nitrogen in decaying leaves and petioles however has been remobilized by the plant during the process of senescence (Williams, 1954). Therefore only a minor amount of nitrogen is released to the soil by this means.

Fairly extensive research has been done on factors affecting the formation and loss of nodules from legume roots. Factors such as fertilizers, season and farm practices are known to affect nodule formation. A lot of work too has been done on longevity and diseases of legume nodules. However, knowledge regarding the biology of nodule decomposition, their carbon dioxide release and also the rate of mineralization of nitrogen from decaying nodules is still lacking. In fact, Thornton (1965) is the only worker who has investigated the surface mycoflora of clover nodules.

Considering the importance of nitrogen in the natural ecosystems and the role of legumes in agriculture, it is surprising that there is not much information on when, where, how and in what form nitrogen is released from decomposing roots and nodules of legumes (Waid, 1974). Such information could have great practical implication to agriculture.

The present study was an attempt to investigate the biology of decomposition of roots and nodules of *Centrosema pubescens*, by considering the biochemical, microbiological and morphological aspects.

The incubation technique was adopted for this study because of the better control of conditions that it provides in the laboratory apart

from being the quickest, cheapest and most reproducible method available.

The biochemical study was mainly on mineralization of N in the soil using treated and untreated (control) soil to study net mineralization over a certain time interval.

The microbiological study was mainly on the mycofloral colonization and succession on decomposing roots and nodules.

The morphological studies were an attempt to study the macroscopic changes of the tissues during the decomposition process.

C h a p t e r 2

General Review of Literature

Chapter 2

Review of Literature

I WORK ON NITROGEN RELEASE FROM DECAYING PLANTS

Much research has been done on nitrogen mineralization to determine the factors affecting it, its methodology and its application to agriculture.

1. Factors

Black (1968) listed 7 main factors affecting mineralization namely, total nitrogen, substrate composition, soil pH, water supply, drying and freezing, temperature and soil-plant interaction. Of these factors, the effects of substrate composition is the most widely studied. Other factors affecting nitrogen release from decaying plants include amount and state of substrate added, whether fresh, dried, chopped or ground (Van Schreven, 1964) and primarily upon the carbon to nitrogen (C/N) ratio of the plant residues (Van Schreven, 1964; Whitehead, 1970; Vallis and Jones, 1973). Plant remnants of low C/N ratio, for example, legume root and nodule residues have been found to increase mineralization and nitrogen production in soils where legumes are grown in association with grasses (Moore, 1962; Birch and Dougall, 1967; Whitehead, 1970; Simpson, 1976). This is because legume contributes much more litter to the soil giving rise to a marked organic horizon and which on mineralization releases considerable amount of nitrate-nitrogen (Birch and Dougall, 1967).

Bartholomew (1965) reported that further decomposition of plant residues are inhibited by the presence of aromatic, non-protein materials such as polyphenols. A similar observation was made by Vallis and Jones

(1973) when they found that the presence of polyphenol compounds like tannin protected the leaf protein in *Desmodium* against microbial decomposition, resulting in a reduction of nitrogen released to the soil.

The mechanisms by which tannin is thought to inhibit decomposition are as follows:-

a) inhibition due to the inactivation of the microbial exocellular enzymes which interfered with the decomposition of large molecular weight compounds, for e.g. proteins (Benoit and Starkey, 1968).

b) tanning of proteins of plant and animal residues to produce complexes that are resistant to decomposition (Basaraba & Starkey, 1966).

c) complexing of tannins with non-proteins to form compounds which are resistant to decomposition (Benoit & Starkey, 1968b).

2. Methodology

Three main methods are used to study nitrogen release from decaying plants, namely the incubation method, the greenhouse pot method and the field plot method. Of these, the incubation method is the most widely used. Iritani and Arnold (1960) used three types of incubation method in their study on nitrogen release by eleven different vegetable residues. These include the flask, the pot and the constant-air-flow technique. All these methods gave similar results. Harmsen and Van Schreven (1955) gave a good discussion on limitations of this incubation technique.

3. Advantages and disadvantages of the incubation technique

The incubation methods have been widely used in evaluating the mineralizing ability of organic substances present in the soil. It is one of the quickest, cheapest and most reproducible method available compared to the other methods of study. The incubation of homogenized samples under optimum laboratory conditions provide standardization of this method, allowing the possibility of obtaining comparable results amongst the workers (Harmsen and Van Schreven, 1955).

There are however several shortcomings regarding this technique. Since the samples under study are incubated under artificial laboratory conditions, the results obtained from such studies will only give the potential mineralizing ability of the substrate. The results will not give the real mineralizing power which prevails under field conditions (Harmsen and Van Schreven, 1955; Bartholomew, 1965). The difference between results obtained by the incubation method of study and that obtained from study under field conditions are very significant (Harmsen and Van Schreven, 1955).

The incubation method however will give reliable results under the following conditions:-

1. By restricting the incubation technique to one soil type, one climatic zone, one farming system and collection of all samples required within one season preferably in Spring (Harmsen and Van Schreven, 1955).
2. Separate determinations of the ammonia, nitrite and nitrate content of the substrate under study (Harmsen and Van Schreven, 1955).

The results obtained from the incubation technique would be difficult to interpret if the results are to be applied to agriculture.

4. Factors investigated

Measurable variables like organic matter content, evolution of carbon dioxide and also the carbon and nitrogen content of the substrate are widely used in mineralization studies. A good correlation between carbon dioxide evolution and degree of mineralization has been reported, for example, Ivarson and Sowden (1959); Allison and Klein (1961) and Van Schreven (1964). The tracer technique was used by Stojanoic and Broadbent (1956) for studying mineralization.

5. Conclusion

Apparently, most of the work on mineralization has been carried out on leaves, litters and residues of plants in general. A fair amount of work has also been done on mineralization of legume plants grown in association with grasses. As far as I know, no work has been done on mineralization of legume roots and nodules *per se*. Therefore more information is required regarding this aspect of mineralization.

II REVIEW OF FUNGAL COLONIZATION OF ROOTS

Introduction

A number of studies has been carried out to determine the mycoflora of various plant roots at different stages of growth and decomposition. Most of the studies were carried out on grasses and legumes (Waid, 1957;

Peterson, 1958; Thornton, 1965; Gadgil, 1965; Mahique, 1966).

1. Methods

In most cases, either roots of healthy plants or buried, excised roots were sampled, and both the direct and indirect methods were used in conjunction with the serial washings of Harley and Waid (1955).

2. Factors involved

Various factors affecting the composition of the root mycoflora have been investigated. These include the age of the plant tissues, state of decomposition, soil type, plant species, position of the roots, climate, season, fungal interactions and also rhizosphere effects. Other aspects studied include route of colonization, rate of colonization and also pattern of colonization.

3. Problems

Studies in this field generally indicate that all the above factors affect the composition of the root mycoflora but the actual mechanisms involved still have to be elucidated, while some methodological problems need to be overcome. This might help to explain or probably eliminate some conflicting observations of some workers on the root mycoflora.

4. Stages of colonization

Mahique (1966) reported that root colonization occurs soon after the roots penetrate the soil, and that colonization takes place a few millimetres behind the root tips (Waid, 1957; Stenton, 1958; Peterson, 1958). Studies on the route of colonization of root surface showed

that the successive lateral colonization from the soil is of greater importance than growth of fungi down the roots (Taylor and Parkinson, 1961). The fungal succession becomes distinct after the initial colonization of the roots and usually starts with the primary colonizers which are the normal root surface mycoflora.

Parkinson *et al.*, in 1963 suggested that the primary colonizers are actually the normal root surface mycoflora which become active in the primary decomposition of root tissues. These primary colonizers or pioneer fungi are actually semi-parasitic in nature and may be able to overcome the host resistance but most appear to have a low degree of competitive saprophytic ability (Waid, 1957). On the other hand, the secondary colonizers have high degree of competitive saprophytic ability (Waid, 1957). As colonization progresses, the cortex becomes occupied by these competing saprophytes which will gradually outnumber the pioneer mycoflora (Waid, 1957). Once the root surface fungi are established, the relative incidence of the different fungi does not change although the amount of mycelium belonging to each fungal species may alter up to the time of plant senescence (Parkinson *et al.*, 1963).

The fungal succession observed on the roots appears to be an expression firstly of the differential response by fungi to the presence of roots and secondly as a result of competition between different fungal forms growing on the roots (Parkinson *et al.*, 1963). The soil type determines which fungal species will become dominant while the host species will determine which fungal species will make up the mycoflora (Parkinson *et al.*, 1963). For example, Thornton (1965) found that *Fusarium oxysporum* was predominant in warm, moist, moderately acid soils. Waid (1974) suggested that fertilizer nitrogen affected the prevalence