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PLANT GROWTH REGULATORS AS A POSSIBLE MECHANISM FOR ALTERING PLANT RHIZOSPHERE MICROBIAL POPULATIONS

The Ohio State University

Ph.D. 1980

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FOR ALTERING PLANT RHIZOSPHERE MICROBIAL POPULATIONS

DISSERTATION
Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

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B.Sc. (Biology) (Agron.) M.S. (Agron.)

* * * * *

The Ohio State University
1980

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INTRODUCTION

Plant-produced growth hormones have been shown to play an integral part in plant morphogenesis from seed germination to plant senescence. These same types of hormones have also been shown to be produced by many typical plant symbiotic, associative and non-associative microorganisms.

Production of growth regulators by associative and free living dinitrogen fixing organisms can alter the growth of root systems (Brown et al., 1986a; Tien et al., 1979) and cause the same morphological changes observed for plants grown under sterile conditions exposed to authentic growth regulators. Symbiotic dinitrogen fixing organisms have also been shown to produce plant type growth regulators (Phillips and Torrey, 1970, 1972), the importance of which is less clear.

Competition for nodule sites by Rhizobium on the host legume has been studied over the last several decades. Most of these studies centered on R. japonicum and indicated differences in competitive ability (for nodule sites) between Rhizobium strains of the same species (Cauldwell, 1969). The mechanism conferring a competitive advantage to one strain vice another has yet to be understood.

There is very little indication in the literature concerning the effect of plant produced growth hormones on the stimulation or suppression of the rhizosphere/rhizoplane microbial population. This study was designed to investigate the effect of plant-type growth regulators on growth of symbiotic, associative and non-associative
dinitrogen fixing bacteria and to determine if these hormones could influence competitiveness because of sensitivity or lack of sensitivity to growth regulators, alone or in combination.
Plant/Microbial Interactions

The number and type of microorganisms found in a soil system can vary greatly depending on such factors as depth, moisture content, nutrient status, organic matter content, and in particular, the proximity to roots. The increased population of microorganisms around the root can be attributed to the increased availability of organic substrates as a result of root exudates, sloughed off cells, etc., as well as numerous soil properties (Dommergues, 1978).

Microbial populations are quickly established on the developing root systems. Many of these organisms do not remain superficially associated with the root but embed themselves in the outer layer (epidermis) of root tissue (Cambell and Rovira, 1973; Greaves and Darbyshire, 1972; Rovira and Cambell, 1974). Fungi have also been found to establish very rapidly on and in root systems, forming stable colonies that cannot be removed by washing (Parkinson, 1965; Waid, 1974).

There appears little doubt that the rhizosphere microflora play a fundamental role in plant nutrition. Inorganic nutrients are solubilized by microbially mediated breakdown of minerals and organic matter (Arrieta and Grez, 1971; Duff et al., 1963). Microbial populations have been shown to play an important role in availability of phosphorus (Agnihortri, 1970; Berea, 1976; Greaves and Webley, 1965; Katznelson et al., 1962; Katznelson and Bose, 1959), calcium (Jackson and Voigt, 1971), and many trace elements (Barber and Lee, 1974; Jones, 1972; Loutit and Brooks, 1970).

Root Exudates

The subject of plant root exudates has been studied extensively (Rovira, 1969; Hale et al., 1971). Sources of root exudates are translocated photosynthates, aromatic and aliphatic acids, amino acids, phenols and
proteins. The composition of exudates from within species as well as between seed and seedlings of the same species are different (Vancura and Hanzlikova, 1972). Vancura and Hanzlikova (1972) observed more reducing compounds in seedling root exudates of barley, bean, cucumber and wheat than from their seeds. They also observed that seed exudates of bean contained five keto sugars while seedling exudates (of bean) contained only fructose. The predominant monosaccharide in bean seed was galactose whereas glucose, arabinose, fructose, etc. were found in seedling root exudates. Kovacs (1971) found larger quantities of free and polymeric aromatic acids in seed exudates of barley, cotton and pea than previously reported.

A. Site of Root Exudate Release

Rovira (1973) and others (McDougall, 1968; McDougall and Rovira, 1965, 1970) used C-14 labelled photosynthates of wheat and determined that 33% of root exudates occurred at the basal region of roots. They found that the majority of the radioactivity came from the tips of the developing lateral roots - the major area being the zone of elongation. Egeraat (1975b) found similar sites for ninhydrin positive compounds of pea seedlings.

Egeraat (1972) studied root exudates of sterile pea plants in an attempt to identify the "factor" involved in stimulating the pea symbiont R. leguminosarum. He identified several ninhydrin-positive compounds of which the amino acid homoserine was quantitatively most important. R. leguminosarum was stimulated by homoserine whereas R. trifolii and R. phaseoli were inhibited in growth when homoserine was the sole source of carbon and nitrogen. In further investigations
Egeraat (1975a, 1975b) found that the tips of the main and lateral roots as well as the site of lateral root initiation were sites of ninhydrin-positive exudates. An unknown compound labelled "Y", isolated from the first 2 mm slice of the root tip, was quantitatively the most prevalent isolate. Homoserine was more prevalent in the second 2 mm slice.

**Rhizobium in the Rhizosphere**

As the rhizosphere has much more intense microbial activity than non-rhizosphere soil, microbial interactions become significant in the ability of a specific microorganism to become established in the rhizosphere. This biological control, or "biological buffering", is the basis for control of plant pathogens at the rhizosphere level (Baker and Cook, 1974). Of particular interest is the competitiveness of associative N\(_2\) fixing microorganisms.

Interactions between the legume root and Rhizobium cells occur at the root surface (rhizosphere/rhizoplane). The literature indicates that specific stimulation of Rhizobium by the host legume does occur (Brown et al., 1968; Egeraat, 1972; Nutman, 1962, 1965), implying that this is part of the initial infection process. On the other hand, evidence also exists that legumes other than the host legume enhance the growth of heterologous Rhizobium species (Tuzimura and Watanabe, 1962). Several grass species of Graminae have also been noted to stimulate Rhizobium (Robinson, 1967; Rovira, 1961; Tuzimura and Watanabe, 1967).

A. **Rhizobium-Host Root Specificity**

The symbiosis between the legume host and Rhizobium is characterized by a high degree of host specificity which has become the basis for distinguishing Rhizobium species. The mechanism for this recognition
between the host legume and specific *Rhizobium* strain has been the subject of rather intense investigation. As mentioned previously, there appears to be an enhancement of effective strains in the rhizosphere/rhizoplane of the host legume. Munn (1968) indicated that plants grown in liquid culture accumulated *Rhizobium* on the host root surface in the first few hours after inoculation in greater number than could be expected based on specific bacterial growth rate. These data along with other inferences led several investigators (Currier and Strobel, 1976, 1977; Dazzo et al., 1976) to propose that specific chemotaxis is responsible for *Rhizobium* recognition at the legume root surface. The concept of chemotactic recognition eventually was superceded by the lectin theory.

**B. Lectin Recognition**

Lectins are proteins, or glycoproteins that bind to cell surfaces via specific oligosaccharide determinants (Lis and Sharon, 1973). They were formerly called phytohemagglutinins by virtue of their ability to agglutinate red blood cells. Lectins are abundant in the seeds of many legumes constituting up to 10% of the total protein (Liener, 1976). Lectins are also known to occur on legume roots (Bhuvaneswari and Bauer, 1978; Lippincott and Lippincott, 1978; Lis and Sharon, 1973), but are poorly characterized as to their identity with seed lectins (of the same plant), their location on the root system, and particularly their presence at infection sites (Schmidt 1979).

The possible relationship of lectin binding to the specificity of legume-rhizobia interaction was first reported by Bohlool and Schmidt (1974). They used the fluorescent antibody technique to show that soybean lectin was bound to most *R. japonicum* strains (22 of 25 strains studied) capable of forming nodules and not to *Rhizobium* spp incapable of forming nodules on
soybeans. Since this initial data, several other investigators have shown that other legume lectins bind specifically to their homologous rhizobial symbiont (Dazzo and Hubbell, 1975; Kamberger, 1978; Solheim, 1975; Wolpert and Albersheim, 1976). This led to the hypothesis that recognition may involve binding of rhizobial cell to lectins through unique carbohydrates on the surface of the Rhizobium cell.

Electron microscopy appear to show a common feature of attachment of Rhizobium to the root hairs of the host. "The adherent rhizobial cell is surrounded by a fibrillar capsule in contact with electron-dense globular aggregates normally having a high affinity for the outer periphery of the legume root hair" (Dazzo 1979). Dazzo and Hubbell (1975) as well as others (Bishop et al., 1977; Dazzo and Brill, 1979; Dazzo et al., 1978) suggested that selective adherence (host to specific rhizobial symbiont) appears to be initiated by a "specific crossbridging of antigenically related saccharide determinants" that appear to be on both the Rhizobium and the cell wall of clover root hair and appears to be a multivalent host-coded lectin called "trifoliin". The ability of R. trifolii to bind to the host plant could therefore be regulated by the plant's ability to accumulate "trifoliin" on the root surface. Dazzo and Brill (1973) showed that NH$_4^+$ or NO$_3^-$ in the rooting media regulate the "trifoliin" level on clover root hairs and the apparent ability to bind to these surfaces.

Capsular polysaccharides (Bal et al., 1978; Calbert et al., 1978; Dazzo and Hubbell, 1975), lipopolysaccharides (Kamberger, 1978; Wolpert and Albersheim, 1976), and a cell wall-associated glycogen (Planque and Kijne, 1977) have been identified as the lectin receptors on the rhizobial cell. Recent work with mutant strains of Rhizobium have identified both
lipopolysaccharides and exopolysaccharides as cell surface components im-
portant to nodulation of legumes (Maier and Brill, 1978; Saunders et al., 1978).

The lectin concept provides insight into host-symbiont recognition
but does not define competitiveness between Rhizobium species capable of
infecting a host legume.

C. Competition for Nodule Sites by Rhizobium

Competition for nodule sites by Rhizobium on the host legume has
also been studied extensively over the past several decades. Most of
these studies centered on R. japonicum and indicated differences for
nodule sites between Rhizobium strains of the same species (Caldwell,
1969; Johnson and Means, 1964; Means et al., 1961). Caldwell and
Vest (1968) and Caldwell and Weber (1970) conducted experiments for
3 years on Codorus silt loam soil containing a naturalized population
of R. japonicum (strains 110, 122 and 125). In the former study
Caldwell and Vest determined that soybean genotypes grown successive
years in different fields, showed significant differences in their
acceptance of R. japonicum strains of specific serogroups. Soybean
genotypes which were genetically related showed similar distributions
of R. japonicum in their nodules. In the latter study, Caldwell and
Weber studied planting date and its effect on nodulation. Serogroup
110 and the mixture of strains 122 and 125 were found to be most
effective at early planting dates. They concluded that variation in
serological reaction due to planting date was influenced by environ-
mental conditions affecting the Rhizobium.

Skrdleta and Karimova (1969) studied the competition between 2
strains (D216 and D344) of R. japonicum in ratios of (D216:D344) 1:60 and
5:3. Strain D216 was more competitive for nodule sites than strain D344
irrespective of the ratio used.
Johnston and Beringer (1976) conducted experiments with mixed inoculum of efficient and inefficient strains of *R. leguminosarum*. They observed that the inefficient strain was more competitive for nodule sites and negatively influenced the nodulation of the efficient strain in the same root system.

Roughley et al. (1976) studied competition of 4 strains of *R. Trifolii* with naturalized strains for nodule sites of *T. subterraneum* in 5 locations in Australia. They found that, in *Rhizobium* free soils, the inoculum strains formed 100% of the nodules the first year. During the second year, serologically unrelated strains invaded the plots, and by the third year they formed the highest percentage of nodules. In soils with a natural population of *Rhizobium*, the inoculum strains were unable to compete for nodule sites.

Russel and Jones (1975) used immunofluorescence to study nodulation by efficient and inefficient strains of *R. trifolii* on *T. ripens*. They observed a tendency for the inefficient strain to produce a higher number of nodules in high pH soils. The efficient strain produced a higher number of nodules at a lower pH.

Labandera and Vincent (1975) evaluated two strains of *R. trifolii* (one highly efficient strain and one inefficient strain) and their competitiveness for nodule sites on *T. subterraneum* and *T. ripens*. Within a mixed inoculum of both strains, the efficient strain was less competitive for nodule sites.

Boonkerd et al. (1978) studied the introduction and competitiveness of three strains of *R. japonicum* (62, 76 and 110) in a rhizobia-populated field using a seed-applied inoculum at a normal rate and 10 x the normal rate, and liquid inoculum at normal rate, 10 and 100 times the normal rate.
applied over the seeds in the row. They observed that inoculation by either method, or with any rate or strain, did not affect nodulation or healthy plant growth. Recovery of strains 62 and 110 increased at 10 and 100 times the initial liquid inoculum rate. Peat inoculum at 10 times the initial rate, did not significantly change the serogroup distribution in the nodules. Strain 76 appeared to be less competitive than indigenous strains at any rate or method of inoculation.

Competition between strains for nodule sites has long been observed. The mechanism conferring a competitive advantage to one strain versus another has yet to be elucidated.

**Plant Derived Indoleacetic Acids**

Indoleacetic acid (IAA) was first purified from plants by Kogel and Kostermans in 1934 and Thimann in 1935. Since that time many plant tissues and organs have been shown to synthesize auxins. Auxins have been shown to be synthesized in grass coleoptile (Schlieng et al., 1977; Tientzeha and Dorfflin, 1977; Went, 1928; Zarra and Masuda, 1979), apical meristems and basal internodes (Gunckel and Thimann, 1949) and embryos (Hemberg, 1955; Luckwill, 1949; Nitsch, 1950). Expanding cells of lengthening internodes and expanding leaves of higher plants also have been shown to produce large amounts of auxins (Steeves and Briggs, 1960; Westmore and Jacobs, 1953) as well as actively growing root systems (Audus, 1972; Thimann, 1937).

Transport of auxin within the plant is considered to be polar (Cande and Ray, 1976; Fry and Wangema, 1976; Jacobs, 1977; Tanaka and Uritani, 1979) and is inferred to be an active transport process (Hertel and Leopold, 1963). Movement of auxin is generally considered to be basipetal in leaves and stems (Jacobs, 1977). In roots there is
general movement towards the root tip (Pernet and Pilet, 1979), however
movement away from the tip has been reported (Nagao and Ohwaki, 1968)
as well as in both directions (Hillman and Phillips, 1970).

Indoleacetic acid has been implicated as the chemical compound
controlling cell elongation (Dayanand et al., 1976; Edwards and Scott,
1977; Patel et al., 1978; Sakurai and Masuda, 1978) as well as apical
dominance, fruit set and fruit growth, tuber and bulb formation, seed
germination and geotropism (Leopold and Kriedemann, 1975).

A. Mode of Action of Indoleacetic Acid

Indoleacetic acid is intimately involved with rapidly dividing
cells and has an effect on plasticity and elasticity of cell walls,
cytoplasmic viscosity, protoplasmic streaming, respiration rate, meta-
bolic pathways, changes in oxidative states, content of nucleic acids
and the activity of many enzymes (Leopold and Kriedemann, 1975).

An auxin binding site has been identified on the endoplasmic
reticulum of corn coleoptile microsomes (Batt and Venis, 1976; Ray,
1977). The apparent binding specificity at this site for natural and
synthetic auxins strongly implicates the endoplasmic reticulum as a
primary site for auxin activity (Ray et al., 1977). Cross and Briggs
(1978, 1979) were able to extract an auxin-binding protein of 80,000
daltons from coleoptile and leaves of Zea mays.

Indoleacetic acid also has some influence on the activity and
functions of other growth regulators. IAA has been shown to rapidly
induce ethylene production (Franklin and Morgan, 1978). The combination of
IAA + ethylene has been shown to induce cell elongation in rice
coleoptiles (Katsura and Suge, 1979) and aquatic plants (Cookson and
Osborne, 1978), as well as influence adventitious root formation and
hypercotyl hypertrophy in flooded sunflower plants (Wample and Reid, 1979). IAA and cytokinins have been shown to effect leaf blade hyponasty (Hayes, 1978). IAA and abscissic acid (ABA) have been shown to influence RNA protein synthesis in Jerusalem artichokes (Monocha and Dibona, 1979). IAA and gibberellins appear to be intimately involved with differentiation of primary phloem fibers (Aloni, 1979) and hypocotyl elongation in cucumber seedlings (Katsumi, 1976) etc.

B. Microbial Production of IAA

The ability of the soil microflora to produce IAA has long been recognized. Krasil'nikov (1958) studied 192 bacterial culture isolates from Russian soils and found 77 cultures produced auxin-like substances. Schmidt and Starkey (1951) found 99 out of 150 species of bacteria and actinomycetes studied produced auxin-like structures. Associative and free living dinitrogen fixing organisms have been shown to produce IAA (Azcon and Berea, 1975; Breckenridge and Knowles, 1970; Brown and Burlingham, 1968; Brown and Walker, 1970; Kefford et al., 1960; Phillips and Torres, 1970; Thimann, 1936). Production of indoleacetic acid by soil fungi has also been reported (Blok, 1973; Buckley and Pugh, 1971; Darbyshire and Greaves, 1971; Gruen, 1959; Gunasekaren and Weber, 1972; Posthumus, 1973). Gruen (1959) indicated 77 of the 81 different species of fungi investigated produced auxin. Mycorrhizae have also been shown to produce auxin (Slankis, 1973).

Investigation of auxin-like activity in soil was first reported by Stewart and Anderson in 1942. Hammence (1944, 1946) found 0.0025-0.008 mg IAA per 100 grams of soil. Wainwright and Pugh (1975) found no indole auxin activity in soil but noted a phenolic acid with auxin-like activity.
Plant Derived Gibberellins

Gibberellins are diterpenoids composed of four isoprene units most commonly arranged to form 3 rings with an additional lactone bridge. There are other plant derivatives that show gibberellin-like activity e.g. steviol (Valio and Rocha, 1975) which is structurally identical to B-hydroxykaurenoic acid and structurally resembles gibberellins (Raddat et al., 1963). Steviol was initially thought to be a precursor to gibberellins (Raddat, 1968). Later research indicated that steviol functions as a precursor for C-13 hydroxygibberellin and not as a gibberellin analog (Alves and Ruddat, 1979). Other plant derivatives cited for gibberellin-like activity are kaurenoic acid (Katsumi et al., 1964) and phaseolic acid isolated from bean plants (Redemann et al., 1968).

The biosynthesis of gibberellins is thought to be via the isoprenoid pathway of acetate metabolism (incorporation of acetate into gibberellin via melvalonic acid) (Birch et al., 1959; Cross, 1968; Lang, 1970; West and Fall, 1972). There is general agreement that interconversions of gibberellins, from one active form to another, can occur within the plant (Graebe et al., 1965; Reid et al., 1972).

The major site of gibberellin synthesis within the plant appears to be the apex of the stem (Jones and Phillips, 1966; Lockhard, 1957) and in particular the leaf primordium in the apex rather than the meristem itself (Humphries and Wheeler, 1964; Jones and Phillips, 1966). Gibberellin biosynthesis has also been indicated to occur in embryos (Palég, 1960) cotyledons (Garcialu and Guardiol, 1978; Sebanek, 1965), fruits (Baldev and Lang, 1955; Ram and Pol, 1979) and seeds (Jarvis and Wilson, 1977; Vonschic, 1979; Weaver and Pool, 1965). Initially roots were thought to
be a major site of gibberellin synthesis (Carr et al., 1964). Crozier and Reid (1971) proposed that gibberellins were synthesized in the shoots as GA$_{19}$, translocated and converted to GA$_1$ in the roots and then retranslocated back to the stem as required.

Translocation of gibberellins within the plant was initially thought to be non-polar (opposite that of auxin) (Chlor, 1967; Hertal et al., 1969). Later research indicated that gibberellins did in fact display polar movement (Jacobs and Kaldewey, 1970; Jacobs and Pruett, 1973). Translocation of gibberellins seems to take place in either the phloem (Hoad and Bowen, 1968) or xylem (Carr et al., 1964).

A. Regulatory Role of Gibberellins

The main regulatory role of gibberellins in plant growth processes appears to be involved with stem elongation (Adams et al., 1975; Katsumi and Kazama, 1978; Montague and Ikuma, 1975, 1978; Suttle and Zeevaart, 1979) since roots and leaves appear to respond weakly to additions of gibberellins (Bhatt et al., 1976). Other major regulatory roles attributed to gibberellins are overcoming dormancy in seeds (Jarvis and Wilson, 1977; Kahn et al., 1957; Smith and Rappaport, 1961; Vonscher, 1979), and hydrolysis of starch reserves in germinating seeds (Garcialu and Guardiol, 1978). The literature also indicates gibberellins influence in overcoming cold requirement and plants sensitive to photoperiods and inducing them to flower, determining sex in flowers, stimulate fruit set in some species, rate of fruit growth and ripening and unrolling of developing leaves amongst others. Neumann and Janossy, (1979) using electron microprobe analysis indicated that gibberellins increase calcium and silica concentrations in elongating cells. They found that application of gibberellins increased the concentration of
calcium of cell walls (after a two hour lag) and silica (immediately). Previous literature (Muller et al., 1974; Soni et al., 1972) did not support their findings. The authors felt that methodology had not allowed previous investigators to observe the increases of calcium and silica as indicated.

B. Mode of Action of Gibberellins

The regulatory role of gibberellin seems to be involved with both cell division (Sachs et al., 1958, Sachs, 1963) and cell elongation without cell division (Haber and Luippold, 1960; Haber et al., 1969, Katsumi and Kazama, 1978). Gibberellin appears to enhance messenger RNA synthesis (Dzhokhad and Goglidze, 1977; Wasilews and Kleczkow, 1976; Wielgat and Kleczkow, 1974).

C. Microbial Synthesis of Gibberellins

Production of gibberellin-like compounds have been reported for a variety of rhizosphere fungi (Darbyshire and Greaves, 1971; Hussain and Vancura, 1970; Kampert et al., 1975; Kazaryan and Agadzhanyan, 1971, 1973; Strzelcz et al., 1975; Turner et al., 1978), Azotobacter (Azcon and Berea, 1975; Barea and Brown, 1974; Lee et al., 1979; Vancura, 1961), Azospirillum (Tien et al., 1979), phosphate solubilizing bacteria (Barea et al., 1976), Rhizobium (Sanchez-Calle, 1976), as well as blue green algae (Gupta and Agarwal, 1973; Taylor and Wilinso, 1977).

In studies by Jackson et al., (1964) Brown et al., (1968) and Azcon and Berea (1975) physiological changes in tomato roots caused by Azotobacter present in the rhizosphere could be duplicated by using authentic gibberellic acid, leading to the speculation that microbially produced gibberellin-like compounds were the active principle in the observed physiological changes. It is known however, that tomatoes
are extremely sensitive to growth regulators. Similar studies using axenic barley seedlings could not duplicate the results of the tomato studies (Lynch and White, 1977). Brown (1972), however, did show stimulation of wheat seedlings to exogenously applied gibberellic acid (GA₃) and indoleacetic acid (IAA). Tien et al., (1979) investigated the effects of authentic plant growth regulators as compared to those produced by *Azospirillum brasilense* on the growth of pearl millet. Authentic gibberellin increased production of lateral roots. This increase was also noted when pearl millet was grown in association with *A. brasilense*. They concluded that growth regulators (IAA, GA₃ and cytokinins) produced by *A. brasilense* in association with pearl millet were responsible for the observed changes in root morphology.

**Plant Derived Cytokinins**

Miller et al., (1955) separated the first known stimulant of cell division from herring sperm DNA which was subsequently named kinetin. The structure was found to be 6-furfurylaminopurine. Kinetin appears not to be a natural component of plants. Letham et al., in 1964, identified the first cytokinin hormone from corn seeds: the compound was called zeatin. Since this initial discovery by Letham, several other purine cytokinins have been isolated from plants, animals as well as microorganisms (Hall, 1973; Skoog and Armstrong, 1970).

**A. Distribution of Cytokinins in Plants**

Cytokinins have been isolated from plant extracts of embryos and young fruits (Davey and Van Staden, 1978, 1979; Smith and Van Staden, 1979; Steward and Shantz, 1956; Van Staden and Stewart, 1975). Cytokinins occur in relatively high concentrations in root tips (Weiss and Vaadia, 1965) and in bleeding sap (Kende, 1965) indicating cytokinins produced in
the root are translocated, in the transpiration stream, to leaves and other aerial parts of the plant. Engelbrect (1972) and Wareing et al.,(1977) working with *P. vulgaris* and *Xanthium strumarium*, respectfully, also indicated that leaves depend on translocation of root produced cytokinins. Others (Van Staden, 1976a, 1976b; Wang et al., 1977) have also shown cytokinin derivatives present in the leaves of various plants. Vonk (1979), however, showed that leaves of yucca plants, whose roots were removed, produced cytokinins, and therefore were not dependent on root synthesized cytokinins. Short and Torrey (1972) found a very rich source of cytokinin in the first millimeter of the root tip of pea plants. In later studies (Torrey and Feldman,1977), this region of the root tip was isolated and designated the "quiescent center".

Synthesis of cytokinins in plants appears to occur via substitution of side chains onto an adenine moiety (Hall,1973; Skoog and Armstrong, 1970). The five carbon side chain of many of the amino purines suggests that they come from an isoprene source (Peterkovsky,1968).

B. Regulatory Role of Cytokinins

The initial role attributed to cytokinin-like compounds was cell division. However with further investigations into the role of cytokinin in plants, it became obvious that cytokinins participate in regulation of growth and differentiation, and in the various stages of plant development. Cytokinins have been shown to enhance RNA and DNA synthesis (Kannanga and Booth,1979; Patau et al.,1957; Romanko et al., 1978; Romanko and Selivank,1977; Selivank et al.,1976; Shininge and Polley,1977) as well as mitosis and cytokinesis (Pandey et al.,1978; Torrey,1961). Other roles attributed to cytokinins are delayed leaf senescence (Kuhnle et al.,1977; Richmond and Lang,1957),
protein synthesis (Klambt, 1976; Maass and Klambt, 1977; Muren and Fosket, 1977; Parthier and Wollgiehn, 1961) and mobilization of elements (Müller and Leopold, 1966) to name just a few.

C. Mode of Action of Cytokinins

Treatment of roots with exogenously applied cytokinins results in a large increase in RNA (Guttman, 1956; Kannanga and Booth, 1979). Samuels (1961) indicated that fasciation and modified growth of pea seedlings closely resembling the symptoms of infection with Corynebacterium fascians could be induced by soaking seeds in high concentrations of kinetin. Armstrong et al., in 1976, was able to isolate and identify an active cytokinin-like compound from C. fascians grown in liquid culture, indicating the possibility of cytokinin involvement in pathogenic infection of host plants.

As mentioned previously, cytokinins influence RNA, DNA and protein synthesis. Others (Dravnicks et al., 1969; Karavaik et al., 1975; Penner and Ashton, 1967; Steinhart et al., 1964) have indicated that cytokinins are involved with formation of specific enzymes. A more indirect mode of action of cytokinin may be concerned with regulation of synthesis of other growth hormones. Cytokinins may increase plant content of auxin (Hall, 1973; Nember, 1972; Skoog and Armstrong, 1970), gibberellin (Loveys and Wareing, 1971) and ethylene (Fucks and Liberman, 1968).

Cytokinins have been extracted from soil (Van Staden and Dimolla, 1976). They were able to extract cytokinins from soil supporting growth of species of Pinus, Corya and Acacia, all of which have root systems that are in symbiotic association with soil microorganisms. They were unable to show cytokinin activity from soil taken from grass pasture.
D. Microbial Production of Cytokinins

The production of cytokinins by microorganisms was reviewed by VanAndel and Fuchs (1972). At that time 11 bacteria and 4 fungi were indicated to produce cytokinin-like compounds. Since their review, cytokinin-like extracts have been reported from cultures of Agrobacterium (Upper et al., 1970), Azotobacter (Azcon and Berea, 1975), Bacillus (Coppola et al., 1971), Lactobacillus (Phillips and Torrey, 1970, 1972), Rhizobium (Phillips and Torrey, 1970, 1972), Corynebacterium (Armstrong et al., 1976), phosphate solubilizing bacteria (Berea et al., 1976), mycorrhizal fungi (Miller, 1967), Rhizopogon (Crafts and Miller, 1974; LaLoue and Hall, 1973), Taphina (Johnston and Trione, 1974), phytopathogenic fungi (Viazrova, 1975) and Schizosaccharomyces (Ashrof et al., 1976).

There is very little evidence in the literature to indicate that production of cytokinins by soil or rhizosphere/rhizoplane microbial populations has a major impact on plant growth. Microbially produced cytokinins can be implicated as a probable cause of observed root morphological changes (Armstrong et al., 1976; Azcon and Berea, 1975; Tien et al., 1979) when comparing microbial versus authentic hormone effects. The importance and full implications of microbially produced cytokinins has yet to be resolved.
MATERIAL AND METHODS

A. Experiments in Liquid Culture Medium

1. Selected Organisms

The purpose of these experiments was to determine the effects of various concentrations and combinations of plant growth hormones on the growth rate of selected dinitrogen fixing organisms.

Microorganisms used in this study were *Rhizobium japonicum* (strains 110 and 123), *Rhizobium phaseoli* (strains 442 and QA 1062), *Azospirillum brasilense* and *Azotobacter beijerinckii*. Strains were obtained from the culture collection of Dr. Robert H. Miller (The Ohio State University, Columbus, Ohio, 43210) with the exception of *A. beijerinckii* obtained from American Type Culture Collection (ATCC 12301 Parklawn Drive, Rockville, Md. 20852, No. 19360).

2. Preparation of Media

Media used throughout this experiment was soil extract-mannitol-yeast extract (SMY) (ATCC Media 111, ATCC Catalogue of Strains, 13th edition, 1978) (Appendix A) except where noted. The media was made up with 80% of the distilled water listed in Appendix A. Twenty ml were dispensed into sidearm flasks (125 ml and/or 300 ml) and autoclaved at 121° C for 20 minutes. Organisms used in the experiment were grown in 25 ml of sterile SMY media, in a rotary shaker (200 rpm) (New Brunswick Scientific Co., New Jersey Model G-25) at 28-30° C. When the cultures were in late logarithmic growth, 0.1-0.2 ml of the culture was used to inoculate the sidearm flasks containing the various concentrations and combinations of growth regulators.
Azospirillum was cultured in medium described by Okon et al., (1977) (Appendix B). Twenty-five ml of media was inoculated with *A. brasilense* and incubated at 33±2°C until late logarithmic growth and then used as previously described to inoculate sidearm flasks.

3. Preparation of Growth Regulators

Growth regulators used in this experiment were indoleacetic acid (ICN Pharmaceuticals, Life Science Group, Cleveland, Ohio 44128, Cat. No. 102037), gibberellic acid (75% potassium salt, ICN Cat. No. 100288), and kinetin (ICN Cat. No. 102117). Solutions of the growth regulators were made in the following manner:

**Indoleacetic acid (IAA)** - 0.05 g of authentic IAA was placed in a 50-ml volumetric flask containing approximately 0.5 ml ethanol (95%). The dissolved IAA was made up to volume, pH adjusted to 6.8-7.0 and filter sterilized through a 0.2 μm sterile filter (Nalgene Filter Cat. No. 120-0020, Sybron/Nalge, Rochester, N. Y., 14602). The IAA was transferred to a sterile 50 ml test tube. Appropriate dilutions were made, with sterile distilled water, to provide enough IAA in a 5 ml aliquot (2.5 ml for mixtures of growth regulators) to give a final concentration of 10, 50 and 100 μg ml⁻¹ of each growth regulator at a final volume of approximately 25 ml.

**Gibberellic acid (GA₃)** - 0.06 g of authentic GA₃ (75% potassium salt) was dispensed into a 50-ml volumetric flask containing approximately 0.1 g NaHCO₃. The GA₃ was dissolved in a small aliquot of distilled water and then made to volume. The pH was adjusted to 6.8-7.0 and filter sterilized. The procedures, dilutions, etc., are the same as those described for IAA.

**Kinetin (Kin)** - 0.05 g of authentic kinetin was weighed out and dissolved in a 50-ml volumetric flask containing 1 ml of 0.5 N HCl.
The Kin was made up to volume with distilled water and filter sterilized. The pH was not adjusted because Kin would precipitate above pH 3.0. Procedures, dilutions, etc., are similar to those described for IAA.

4. Treatments

Treatments used throughout this experiment were as follows:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (ug/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA</td>
<td>0, 10, 50, 100</td>
</tr>
<tr>
<td>GA_3</td>
<td>0, 10, 50, 100</td>
</tr>
<tr>
<td>Kin</td>
<td>0, 10, 50, 100</td>
</tr>
<tr>
<td>IAA/GA_3</td>
<td>(Control)</td>
</tr>
</tbody>
</table>

Each treatment was at a concentration of 0 (control), 10, 50 and 100 µg ml⁻¹ of each growth regulator. Duplicate flasks were used for each treatment. Turbidometric readings were made on a Klett-Summerson Photoelectric Colorimeter (Model 800-3, Klett MFO Co. N. Y., No. 42 blue filter) at 4-6 hour intervals until stationary growth phase was reached.

Azospirillum was cultured in media (Appendix B) containing no yeast extract and utilizing concentrations of growth regulators of 0 (control), 1, 10 and 50 ug ml⁻¹. A second experiment was conducted incorporating the minimum rate of yeast extract required for growth (0.005 g l⁻¹).

B. Soil Experiments

1. Soil Preparation

The soil used in this experiment and subsequent experiments was a Miamian silt loam (Typic hapludalf, fine, mixed mesic). The surface 15 cm (litter removed) was collected from the Ohio State University farm, passed through a 3 mm sieve and stored at 4° C. Chemical and physical properties are noted in Table 1.
Table 1. Physical and chemical properties of experimental soil (Miamian silt loam).

<table>
<thead>
<tr>
<th>Particle Size Analysis</th>
<th>Exchangeable Cations</th>
<th>Total Nitrogen</th>
<th>Organic Matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td>Silt</td>
<td>Clay</td>
<td>pH(2:1)</td>
</tr>
<tr>
<td>24.7</td>
<td>56.9</td>
<td>18.4</td>
<td>6.15</td>
</tr>
</tbody>
</table>
Prior to the experiment 1 kg subsample of the Miamian soil was air-dried and the moisture content determined. Ten grams of soil (dry weight basis) was placed in each of 62 flasks (50 ml erlenmeyer). The flasks were autoclaved at 121° C for 1 hour.

2. Preparation of Growth Regulators

Growth regulators (IAA, GA$_3$ and Kin) were made up as previously described and filter sterilized. Each treatment was prewetted with the indicated growth regulator at either 10 or 100 ug g$^{-1}$ soil (dry weight) basis). Controls were prewetted with sterile distilled water. Flasks were allowed to equilibrate for 24 hours prior to introduction of R. phaseoli.

3. Treatments

Treatments compared in this experiment are the same as those described for part A in this section.

4. Experiment Procedures (R. phaseoli strain 442)

R. phaseoli (strain 442) was grown in 100 ml of smy liquid media on a rotary shaker (200 rpm, 28-30° C) for 48 hours. Turbidity was adjusted to yield approximately $1 \times 10^9$ cells ml$^{-1}$. Cells were centrifuged (International Equipment Co., Boston, Mass. Model PR-2) at 10,800 rpm and washed 3 times in 0.01 M phosphate buffer at pH 7.0 (Appendix C) and resuspended to the initial adjusted volume. One ml of the cell suspension was aseptically transferred into each flask which brought the final moisture content up to the equivalent of 1/3 bar. The moisture content was maintained at 1/3 bar during incubation at 28° C. The population of R. phaseoli (strain 442) was estimated after 0, 1, 2, 3 and 12 days of incubation.
a. Plate count

The soil in each erlenmeyer was removed with a total of 95 ml sterile distilled water and transferred into a sterile 250-ml erlenmeyer containing 10 g of glass beads (6 mm diameter). The flasks were placed on a rotary shaker (200 rpm) for 20 minutes. Decimal dilutions were prepared using sterile distilled water and population of *R. phaseoli* estimated by plating a SMY agar. Each dilution plated was replicated 3 times.

b. *Rhizobium phaseoli* Strain QA 1062

Methodology, incubation and extraction procedures are the same as those described for *R. phaseoli* strain 442 with the following difference. Populations of *R. phaseoli* strain QA 1062 were estimated after 0, 1, 2, 3 and 7 days of incubation.

c. Mixed Cultures of *Rhizobium phaseoli* Strains 442 and QA 1062

Methodology, incubation and extraction procedures are the same as those described for *R. phaseoli* strain QA 1062. Numbers of each strain were determined by counting each strain separately. The ability to distinguish strain 442 from strain QA 1062 is based on differences in colony morphology. Colonies of strain 442 are small (1-2 mm), white and opaque. Colonies of strain QA 1062 are larger (4-5 mm), colorless and translucent.

C. Competition Between *Rhizobium phaseoli* strains (442 and QA 1062 for Nodule Sites

1. Growth Chamber Study

   a. Leonard jar and seed preparation

   A growth chamber study was conducted to ascertain the competitiveness between two strains (442 and QA 1062) of *R. phaseoli* for nodule sites on black beans (*Phaseolus vulgaris* var Osorio, 51052) obtained from Dr. Caio Vidor (Departamento de Solos UFRGS, Caixa Postal 776, 90000, Porto Alegre, RS).
Seeds were surface sterilized by soaking in ethanol (95%) for 5 minutes followed by 5 successive washings with sterile distilled water. Leonard jars, used in this experiment, were filled with vermiculite (Terra-lite, Grace Horticultural Products, Cambridge, Mass. 02140) and 250 ml of minus nitrogen nutrient solution (Appendix D) and sterilized at 121° C for 1 hour.

Four sterilized seeds were planted in each Leonard jar and thinned to two plants per jar after germination. Each treatment was replicated three times.

b. Preparation of R. phaseoli inoculum

The cultures of R. phaseoli (strains 442 and QA 1062) were grown in SMY liquid media (Appendix A) on a rotary shaker (200 rpm 28-30° C) for 48 hours. Turbidity was adjusted on a Klett-Summerson Photoelectric Colorimeter (No. 42 blue filter) to yield approximately $1 \times 10^9$ cells ml$^{-1}$ for each strain. Cells were centrifuged, washed with phosphate buffer and resuspended as previously described. (Part B). Each seed was inoculated with 0.5 ml of the appropriate strain or an equal mixture of the two strains.

c. Growth chamber parameters

Leonard jars were randomly placed in a growth chamber with 14 hours daylength, day/night temperature of 30° C/26° C respectively, and an average light intensity of 54,000 lux.

The N-free nutrient solution (Appendix D) was diluted to 1/5 strength with sterile distilled water and used to water the plants as required.

d. Harvesting

The bean plants were harvested 33 days after planting. The vermiculite was rinsed off the roots and the upper portion of the plant separated
and discarded. The fresh nodules were used for serological
determination.

2. Serological Identification of *Rhizobium phaseoli* Strains From
Excised Nodules

After harvesting the plants from the growth chamber, sixty nodules
where taken, at random, from the mixed *R. phaseoli* treatment and
twenty nodules from each individual strain treatments. The remaining
nodules were left on the roots and stored at -20\(^\circ\) C.

Individual nodules were crushed in small test tubes containing 1 ml
of a sterile 0.85% NaCl solution. They were heated for 30 minutes
in boiling water (Means and Johnson 1968). Agglutination reactions were
carried out in disposable trays (Disposo Trays, Model 240-U-CS clear,
Limbro Chemical Co., Inc., New Haven, Conn.) by adding 2 drops of nodular
antigen to each of 3 depressions across the disposable tray. This was
done for each nodule. Each diluted antiserum (1/20) was dispensed
(1-2 drops) down a designated column. A control column using 0.85% NaCl
was included to check for spontaneous agglutination.

D. Statistical Method Used for Analysis of Results

Regression analysis was used to determine the statistical significance
of differences between treatment means. This was done for each individual
treatment as well as combination of treatments at each concentration.
If initial regression analysis indicated significant means within the
model design, the Waller/Duncan or Duncan's Multiple Range Test, at the
5% level, was used to partition out significant means.
RESULTS

A. The Effect of Plant Growth Hormones on Symbiotic, Associative and Free-living Dinitrogen Fixing Soil Organisms in Liquid Culture.

A series of experiments were conducted to ascertain the effects of indoleacetic acid (IAA), gibberellic acid (GA₃) and kinetin (Kin), alone or in combination, at 4 concentrations, on Azotobacter beijerinckii, Rhizobium phaseoli (strains 442 and QA 1062), Rhizobium japonicum (strains 110 and 123) and Azospirillum brasilense.

1. Azotobacter beijerinckii

Azotobacter beijerinckii was grown in 125 ml sidearm flasks containing soil extract-yeast-mannitol (SYM) media. The media was kept aerated on a rotary shaker (200 rpm) at 28-30°C. Turbidity, measured on a Klett-Summerson colorimeter (No. 42 blue filter), was used as an indication of population changes.

Figs. 1, 2 and 3 shows the results of the individual growth regulators IAA, GA₃ and Kin, respectfully, at 4 concentrations (0, 10, 50 and 100 µg/ml) on growth of A. beijerinckii. Fig. 1 shows a slight retardation of growth with increased concentration of IAA. This suppression of growth of A. beijerinckii was temporary and by the 36th hour there was no apparent difference between treatments. The Waller/Duncan K-Ratio Test used to test for significantly different means at the 5% level indicated that there were no significant differences between the growth of A. beijerinckii at the 0 and
Fig. 1  The effect of IAA at 4 concentrations on the growth of A. beijerinckii in liquid culture.

○ = control, □= 10 µg/ml, △= 50 µg/ml, □= 100 µg/ml

Fig. 2  The effect of GA$_3$ at 4 concentrations on the growth of A. beijerinckii in liquid culture.

○ = control, □= 10 µg/ml, △= 50 µg/ml, □= 100 µg/ml

Fig. 3  The effect of kinetin at 4 concentrations on the growth of A. beijerinckii in liquid culture.

○ = control, □= 10 µg/ml, △= 50 µg/ml, □= 100 µg/ml
10 µg/ml rates at any sampling time. There was however, a significant growth difference between the control medium and 100 µg/ml IAA through the 24th hour after which all means were equal.

There was no influence of GA$_3$ on growth of *A. beijerinckii* (Fig. 2) until 24 hours of growth, after which GA$_3$ increased growth slightly at all concentrations. There was very little difference in growth of *A. beijerinckii* at the 50 and 100 µg/ml rates of GA$_3$, however, growth at these rates were significantly higher than the 10 µg/ml rate or the control. Growth at the 10 µg/ml rate of GA$_3$ was also significantly higher than the control.

Kinetin showed a complete inhibition of *A. beijerinckii* growth at the higher concentrations (50 and 100 µg/ml) (Fig. 3). Growth of this organism at the 10 µg/ml rate was slightly below, but not significantly lower, than the control.

The effect of IAA alone, or in combination with GA$_3$ and Kinetin, on the growth of *A. beijerinckii* at levels of 10, 50 and 100 µg/ml are presented in Figs. 4, 5 and 6 respectively. There was an initial suppression of growth of this organism below the control for all treatments from 16 through 36 hours after which growth within each treatment increased slightly above the control. None of these differences, however, were statistically significant. The same initial retardation of growth of *A. beijerinckii* was also observed for the treatments at the 50 µg/ml level (Fig. 5). The IAA/Kin treatment significantly suppressed the growth of this organism for the duration of the experiment. There was a slight but not significant increase in growth of *A. beijerinckii* with the IAA/GA$_3$/Kin treatments.
Fig. 4 The effects of other growth regulators (GA$_3$ and kinetin) in combination with IAA (each growth regulator at 10 µg/ml) on the growth of *A. beijerinckii* in liquid culture.

@ = Control, ○ = IAA, ○ = IAA/GA$_3$, △ = IAA/Kin, □ = IAA/GA$_3$/Kin

Fig. 5 The effect of other growth regulators (GA$_3$ and kinetin) in combination with IAA (each growth regulator at 50 µg/ml) on the growth of *A. beijerinckii* in liquid culture.

@ = Control, ○ = IAA, ○ = IAA/GA$_3$, △ = IAA/Kin, □ = IAA/GA$_3$/Kin

Fig. 6 The effects of other growth regulators (GA$_3$ and kinetin) in combination with IAA (each growth regulator at 100 µg/ml) on growth of *A. beijerinckii* in liquid culture.

@ = Control, ○ = IAA, ○ = IAA/GA$_3$, △ = IAA/Kin, □ = IAA/GA$_3$/Kin
Klett Reading ($x \times 10^2$)

Fig. 4

Fig. 5

Fig. 6

Time (Hours)
The growth suppression by IAA/Kin was complete and greater at 100 μg/ml than at 50 μg/ml (Compare Fig. 5 with 6). Growth of this organism with other combinations of growth regulators at 100 μg/ml was not significantly lower than the control.

Data from Fig. 3 showed the complete suppression of growth of _A. beijerinckii_ by kinetin at 50 and 100 μg/ml. This suppression was still evident with the IAA/Kin combination at 100 μg/ml (Fig. 6) but not at 50 μg/ml (Fig. 5). These data show that IAA modifies the growth suppressing effect of kinetin on _A. beijerinckii_.

The growth of _A. beijerinckii_ with GA$_3$ along or in combination with other growth regulators (IAA and Kin), at 3 levels are shown in Figs. 7, 8 and 9. GA$_3$ alone, at all concentrations, tended to increase growth of _A. beijerinckii_ above the control. Regardless of concentration, combinations of GA$_3$ with other growth hormones resulted in growth suppression, especially early in the experiment. Later, growth exceeded that of the control medium, but never exceeded growth of GA$_3$ alone. Kinetin did not suppress growth as much in the presence of GA$_3$ as it did in combination with IAA.

Growth of _A. beijerinckii_ in the presence of kinetin alone or in combination with other growth regulators (IAA and GA$_3$) at levels of 10, 50 and 100 μg/ml are shown in Figs. 10, 11 and 12 respectively. Kinetin alone or in combination with IAA (50 and 100 μg/ml levels suppressed growth of _A. beijerinckii_. IAA partially modified the totally suppressing effect of kinetin at the 50 μg/ml level, while GA$_3$, after a slight initial inhibition, modified the effect of kinetin still further and increased growth slightly, but not significantly above the control.
The effects of other growth regulators (IAA and kinetin) in combination with GA₃ (each at 10 μg/ml) on growth of *A. beijerinckii* in liquid culture.

- ⊙: Control
- ○: GA₃
- □: IAA/GA₃
- △: GA₃/Kin
- ■: IAA/GA₃/Kin

Fig. 8

The effects of other growth regulators (IAA and kinetin) in combination with GA₃ (each at 50 μg/ml) on growth of *A. beijerinckii* in liquid culture.

- ⊙: Control
- ○: GA₃
- □: IAA/GA₃
- △: GA₃/Kin
- ■: IAA/GA₃/Kin

Fig. 9

The effects of other growth regulators (IAA and kinetin) in combination with GA₃ (each at 100 μg/ml) on growth of *A. beijerinckii* in liquid culture.

- ⊙: Control
- ○: GA₃
- □: IAA/GA₃
- △: GA₃/Kin
- ■: IAA/GA₃/Kin
Fig. 10

The effects of other growth regulators (IAA and GA3) in combination with kinetin (each at 10 µg/ml) on the growth of A. beijerinckii in liquid culture.

⊙ = Control, ○ = Kin, ◊ = IAA/Kin, △ = GA3/Kin, □ = IAA/GA3/Kin

Fig. 11

The effects of other growth regulators (IAA and GA3) in combination with kinetin (each at 50 µg/ml) on the growth of A. beijerinckii in liquid culture.

⊙ = Control, ○ = Kin, ◊ = IAA/Kin, △ = GA3/Kin, □ = IAA/GA3/Kin

Fig. 12

The effects of other growth regulators (IAA and GA3) in combination with kinetin (each at 100 µg/ml) on growth of A. beijerinckii in liquid culture.

⊙ = Control, ○ = Kin, ◊ = IAA/Kin, △ = GA3/Kin, □ = IAA/GA3/Kin
2. *Azospirillum brasilense*

An attempt was initially made to grow *Azospirillum brasilense* without yeast extract in the medium to eliminate addition of growth regulators in the yeast extract. However, this attempt was unsuccessful, even with growth regulators present. For this reason, a preliminary experiment was conducted to determine the minimum amount of yeast extract required for growth. It was found that 5 mg/l was the minimum amount of yeast extract necessary to support growth compared to 100 mg/l recommended in the original formulation (Appendix B). All subsequent experiments employed 5 mg/l yeast extract in the growth medium. In these experiments growth regulators were at 1, 10 and 50 μg/ml since the literature (Tien et al., 1979) indicated that *A. brasilense* was very sensitive to growth regulators.

The effect of IAA at 4 levels (0, 1, 10 and 50 μg/ml) on the growth of *A. brasilense* is shown in Fig. 13. The growth of *A. brasilense* with 100 mg/l yeast extract, and no growth regulators, was also plotted on all graphs as the upper control (UC) and was included for comparison purposes only. (Statistical analysis did not include the UC for comparison with the other treatments). Growth of *A. brasilense*, at all concentrations of IAA, were above the lower control (LC) (containing 5 mg/l yeast extract) through 40 hours after which the 1.0 and 10 μg/ml levels continued to increase at the same rate as the control. The 50 μg/ml treatment, with IAA, continued to increase but at a lower rate and growth at the end of the experiment was less than the control.

There was little difference between growth of *A. brasilense* with the various concentrations of GA, and the control (LC) throughout the
Growth of A. brasilense in liquid culture with 4 concentrations of IAA.
LC = Control (5.0 mg/1 yeast),
O = 1.0 µg/ml IAA, O = 10 µg/ml IAA
= 50 µg/ml IAA,
UC = Control (100 mg/1 yeast)

Growth of A. brasilense in liquid culture with 4 concentrations of GA$_3$.
LC = Control (5.0 mg/1 yeast),
O = 1.0 µg/ml GA$_3$, O = 10 µg/ml GA$_3$,
= 50 µg/ml GA$_3$
UC = Control (100 mg/1 yeast)

Growth of A. brasilense in liquid culture with 4 concentrations of kinetin.
LC = Control (5.0 mg/1 yeast),
O = 1.0 µg/ml kin, O = 10 µg/ml kin,
= 50 µg/ml kin
UC = Control (100 mg/1 yeast).
experiment (Fig. 16). The Waller/Duncan test, however, did show that growth of *A. brasilense* in the control was significantly lower than all other rates of IAA through the first 40 hours.

All treatments with kinetin increased growth of *A. brasilense* above the control (LC) except for the last two sampling periods at 60 and 64 hours (Fig. 15). The growth stimulation increased with increasing concentration of kinetin. It is interesting to note that growth within the 10 μg/ml level of kinetin was approximately equal to the UC containing 100 mg/1 yeast extract, while growth with 50 μg/ml kinetin was considerably, and significantly higher, than all other treatments, including the UC. This was the first instance where growth of any treatment exceeded the growth of the upper control containing 100 mg/1 yeast extract.

The positive effect on growth of *A. brasilense* by 50 μg/ml kinetin and, as will be seen later, by 10 μg/ml IAA/\(\text{GA}_3\)/Kin and 50 μg/ml \(\text{GA}_3\)/Kin, implies that any plant root system that secretes high concentrations of kinetin alone, or combinations of growth regulators at a lesser concentration, could increase the number of *A. brasilense* intimately associated with the root and thus enhance the benefits of that plant/microorganism association.

Growth of *A. brasilense* with \(\text{GA}_3\) and kinetin in combination with IAA or IAA alone at 1.0 μg/ml, are shown in Fig. 16. Growth within all treatment combinations trended lower than growth observed with IAA alone, but were not different from one another. Increasing the concentration of growth regulators to 10 μg/ml (Fig. 17) increased the growth response of *A. brasilense* above the lower control (LC),
The effect on growth of *A. brasilense* grown in liquid culture in the presence of other growth regulators (GA3 and kinetin) in combination with IAA (all growth regulators at 1.0 µg/ml).

LC = Control (5.0 mg/1 yeast), ○ = IAA, ○ = IAA/GA3, △ = IAA/Kin, □ = IAA/GA3/Kin

UC = Control (100 mg/1 yeast)

---

The growth response of *A. brasilense* grown in liquid culture with other growth regulators (GA3 and kinetin) in combination with IAA (all growth regulators at 10 µg/ml).

LC = Control (5.0 mg/1 yeast), ○ = IAA, ○ = IAA/GA3, △ = IAA/Kin, □ = IAA/GA3/Kin

UC = Control (100 mg/1 yeast)

---

The effect on growth of *A. brasilense* grown in liquid culture with other growth regulators (GA3 and kinetin) in combination with IAA (all growth regulators at 50 µg/ml).

LC = Control (5.0 mg/1 yeast), ○ = IAA, ○ = IAA/GA3, △ = IAA/Kin, □ = IAA/GA3/Kin

UC = Control 100 mg/1 yeast)
for the major portion of the experiments. Growth of *A. brasilense* with IAA/GA$_3$/Kin was significantly higher than all other treatments, including the upper control (UC) through the first 48 hours. The combination of all growth regulators at 10 µg/ml provided a positive synergistic effect on growth of *A. brasilense*. In contrast, growth of *A. brasilense* was reduced by all combinations of growth regulators at the 50 µg/ml level (Fig. 18). Growth stimulation noted for IAA/GA$_3$/Kin, at 10 µg/ml (Fig. 17) had disappeared when all were supplied at the 50 µg/ml rate. The IAA/Kin combination suppressed the growth of *A. brasilense* to the greatest degree, but the decrease was not significantly different from the lower control (LC).

Growth regulators in combination with GA$_3$ had little effect on growth of *A. brasilense* at the 1.0 µg/ml level (Fig. 19). When the concentration of all growth regulators were increased to 10 µg/ml (Fig. 20), greater differences in growth response occurred. Maximum growth occurred in the IAA/GA$_3$/Kin treatment and was greater than all other treatment combinations containing GA$_3$ or GA$_3$ alone. The difference was statistically higher than all other treatments through 52 hours. After 52 hours there were no growth differences. Increasing the concentration of growth regulators to 50 µg/ml (Fig. 21) had little effect on the growth of *A. brasilense* except for the GA$_3$/Kin treatment which increased growth from 16 through 52 hours. It had previously been shown that GA$_3$ at 50 µg/ml (Fig. 14), had not increased the growth of *A. brasilense* above the control and the other GA$_3$ levels. Kinetin had however, at the same level (Fig. 15), greatly increased the growth of *A. brasilense*. Therefore, it seems that
Growth response of A. brasilense grown in liquid culture with other growth regulators (IAA and kinetin) in combination with GA\(_3\) (all growth regulators at 1.0 \(\mu\)g/ml).

LC = Control (5.0 mg/l yeast), \(\bigcirc\) = GA\(_3\), \(\bigcirc\) = IAA/GA\(_3\), \(\triangle\) = GA\(_3\)/Kin, \(\square\) = IAA/GA\(_3\)/Kin

UC = Control (100 mg/l yeast)

The effect on growth of A. brasilense grown in liquid culture with other growth regulators (IAA and kinetin) in combination with GA\(_3\) (all growth regulators at 10 \(\mu\)g/ml).

LC = Control (5.0 mg/l yeast), \(\bigcirc\) = GA\(_3\), \(\bigcirc\) = IAA/GA\(_3\), \(\triangle\) = GA\(_3\)/Kin, \(\square\) = IAA/GA\(_3\)/Kin

UC = Control (100 mg/l yeast)

The growth response of A. brasilense grown in liquid culture with other growth regulators (IAA and kinetin) in combination with GA\(_3\) (all growth regulators at 50 \(\mu\)g/ml).

LC = Control (5.0 mg/l yeast), \(\bigcirc\) = GA\(_3\), \(\bigcirc\) = IAA/GA\(_3\), \(\triangle\) = GA\(_3\)/Kin, \(\square\) = IAA/GA\(_3\)/Kin

UC = Control (100 mg/l yeast)
the stimulation of growth of *A. brasilense* shown for the GA$_3$/Kin treatment in Fig. 21 was attributable to the stimulatory effect of kinetin in this combination.

Growth of *A. brasilense*, in a medium with combinations of growth regulators containing Kin at the 1.0 µg/ml level (Fig. 22), was no different than the lower control (LC). Increasing the concentration of all growth regulators to 10 µg/ml (Fig. 23) increased growth over the lower control (LC) for all growth regulator combinations. The greatest suppression of growth of *A. brasilense* was noted within the IAA/Kin treatment (Fig. 24). Growth with this treatment was significantly lower than with kinetin alone as well as the GA$_3$/Kin treatment. IAA, at the 50 µg/ml level had been shown to inhibit growth (Fig. 13) while kinetin at the same rate stimulated growth to the greatest extent (Fig. 15). In combination (IAA/Kin) the IAA fraction not only negated the stimulatory effect of Kin alone, but also caused a negative synergistic effect with kinetin, depressing growth lower than that observed for 50 µg/ml IAA alone (Fig. 13).

3. *Rhizobium phaseoli* (strain 442)

The growth response of *R. phaseoli* strain 442 to individual growth regulators (IAA, GA$_3$, and kinetin) at 4 levels (0, 10, 50 and 100 µg/ml) are presented in Figs. 25, 26 and 27. Increasing concentrations of IAA resulted in increased growth depression of *R. phaseoli* strain 442 (Fig. 25). After 15 hours the growth response of strain 442 was significantly lower with increasing concentration of IAA. This indicated that *R. phaseoli*, strain 442 was extremely sensitive to even minute quantities of IAA.
The effect on growth of A. brasilense grown in liquid culture with other growth regulators (IAA and GA₃) in combination with kinetin (all growth regulators at 1.0 µg/ml).
LC = Control (5.0 mg/l yeast), ○ = Kin, ○ = IAA/Kin, △ = GA₃/Kin, □ = IAA/GA₃/Kin
UC = Control (100 mg/l yeast)

The growth response of A. brasilense grown in liquid culture with other growth regulators (IAA and GA₃) in combination with kinetin (all growth regulators at 10 µg/ml).
LC = Control (5.0 mg/l yeast), ○ = Kin, ○ = IAA/Kin, △ = GA₃/Kin. □ = IAA/GA₃/Kin, UC = Control (100 mg/l yeast)

The effect on growth of A. brasilense grown in liquid culture with other growth regulators (IAA and GA₃) in combination with kinetin (all growth regulators at 50 µg/ml).
LC = Control (5.0 mg/l yeast), ○ = Kin, ○ = IAA/Kin, △ = GA₃/Kin, □ = IAA/GA₃/Kin, UC = Control (100 mg/l yeast)
R. phaseoli, strain 442 responded differently to GA$_3$ than to IAA (Fig. 26). The growth responses to all concentrations of GA$_3$ were slightly, but not significantly, above the control throughout the experiment.

Growth of R. phaseoli, strain 442 was suppressed with increasing concentrations of kinetin (Fig. 27). All concentrations of kinetin produced a statistically significant suppression of growth except the 10 µg/ml level. Growth of strain 442 with 50 µg/ml kinetin was significantly lower than that observed for the 10 µg/ml level and significantly higher than growth with 100 µg/ml kinetin.

The effects of growth regulators on growth of R. phaseoli, strain 442, in combination with each other at levels of 10, 50 and 100 µg/ml are presented in Figs. 28 through 36. The response to individual growth regulators are not included in the remainder of these growth curves. The growth experiments with the individual growth regulators were done at different times and were therefore not comparable. For comparison, refer to Figs. 25, 26 and 27. The suppression of growth of strain 442, in all combination of growth regulators with IAA, at the 10 µg/ml level for each (Figs. 28, 29 and 30), can be attributed to the IAA. Kinetin, in combination with IAA, modified the suppressing effect of IAA and allowed strain 442 to grow at approximately the same rate as the control. GA$_3$/Kin treatment also allowed growth of strain 442 to approximate that of the control, but with the addition of IAA to this combination (IAA/GA$_3$/Kin), the greatest suppression of growth of strain 442 was observed. Growth of strain 442 with the IAA/GA$_3$
Fig. 25 The effect of IAA at 4 concentrations on growth of *R. phaseoli*, strain 442, in liquid culture.
- ○ = Control, □ = 10 µg/ml, △ = 50 µg/ml, □ = 100 µg/ml

Fig. 26 The effect of GA₃ at 4 concentrations on growth of *R. phaseoli*, strain 442, in liquid culture.
- ○ = Control, ○ = 10 µg/ml, △ = 50 µg/ml, □ = 100 µg/ml

Fig. 27 The effect of kinetin at 4 concentrations on growth of *R. phaseoli*, strain 442, in liquid culture.
- ○ = Control, ○ = 10 µg/ml, △ = 50 µg/ml, □ = 100 µg/ml
Fig. 28 The effects of IAA in combination with other growth regulators (GA₃ and kinetin) on growth of R. phaseoli, strain 442. Each treatment is at 10 μg/ml for each growth regulator indicated.

○ = Control, ○ = IAA/GA₃, △ = IAA/Kin,
□ = IAA/GA₃/Kin

Fig. 29 The effects of GA₃ in combination with other growth regulators (IAA and kinetin) on growth of R. phaseoli, strain 442, in liquid culture. Each treatment is at 10 μg/ml for each growth regulator indicated.

○ = Control, ○ = IAA/GA₃, △ = GA₃/Kin,
□ = IAA/GA₃/Kin

Fig. 30 The effects of kinetin in combination with other growth regulators (IAA and GA₃) on growth of R. phaseoli, strain 442, in liquid culture. Each treatment is at 10 μg/ml for each growth regulator indicated.

○ = Control, ○ = IAA/Kin, △ = GA₃/Kin,
□ = IAA/GA₃/Kin
Fig. 31  The effects of other growth regulators (GA$_3$ and kinetin) in combination with IAA on the growth of *R. phaseoli*, strain 442. Concentration of growth regulators for each treatment are 50 µg/ml. 
- ○ = Control, ○ = IAA/GA$_3$, △ = IAA/Kin, □ = IAA/GA$_3$/Kin

Fig. 32  The effects of GA$_3$ in combination with other growth regulators (IAA and kinetin) on the growth of *R. phaseoli*, strain 442. Each growth regulator indicated for each treatment is at a concentration of 50 µg/ml. 
- ○ = Control, ○ = IAA/GA$_3$, △ = IAA/Kin, □ = IAA/GA$_3$/Kin

Fig. 33  The effects of kinetin in combination with other growth regulators (IAA and GA$_3$) on growth of *R. phaseoli*, strain 442, in liquid culture. Each growth regulator indicated for each treatment is at 50 µg/ml concentration. 
- ○ = Control, ○ = IAA/Kin, △ = GA$_3$/Kin, □ = IAA/GA$_3$/Kin
Effect of IAA in combination with other growth regulators (GA$_3$ and kinetin) on the growth of *R. phaseoli*, strain 442, in liquid culture. Concentrations of each growth regulator indicated for each treatment is at 100 μg/ml.

- ○ = Control, ○ = IAA/GA$_3$, △ = IAA/Kin, □ = IAA/GA$_3$/Kin

Fig. 35

Effect of GA$_3$ in combination with other growth regulators (IAA and kinetin) on growth of *R. phaseoli*, strain 442, in liquid culture. Concentrations of each growth regulator indicated for each treatment is at 100 μg/ml.

- ○ = Control, ○ = IAA/GA$_3$, △ = GA$_3$/Kin, □ = IAA/GA$_3$/Kin

Fig. 36

Effect of kinetin in combination with other growth regulators (IAA and GA$_3$) on growth of *R. phaseoli*, strain 442, in liquid culture. Concentrations of each growth regulator indicated for each treatment is at 100 μg/ml.

- ○ = Control, ○ = IAA/Kin, △ = GA$_3$/Kin, □ = IAA/GA$_3$/Kin
combination at 10 µg/ml approximates the growth curve observed for strain 442 grown with IAA alone at the 10 µg/ml rate (Fig. 25).

The growth curves of *R. phaseoli*, strain 442, with combinations of growth regulators at 50 and 100 µg/ml (Figs. 31-33 and 34-36 respectively) proved to be almost identical in response to, but of greater magnitude than that with 10 µg/ml of each. All treatment combinations containing IAA completely suppressed growth of strain 442. The GA₃/Kin combination allowed intermediate growth of strain 442, significantly lower than the control, but significantly higher than the other treatments containing growth regulators.

4. *Rhizobium phaseoli* (strain QA 1062)

The growth of *R. phaseoli*, strain QA 1062, in liquid culture with 4 concentrations of IAA are presented in Fig. 37. An initial and statistically significant suppression of growth of *R. phaseoli*, strain QA 1062, was observed with the higher (50 and 100 µg/ml) concentrations of IAA. After 29 hours, this suppression changes to a stimulation of growth for all concentrations of IAA. At the end of the experiment, growth of strain QA 1062, at the 10 and 50 µg/ml levels, was approximately equal to, and significantly higher than, growth within all other treatments. Growth with the 100 µg/ml level of IAA was significantly lower than growth with the 10 and 50 µg/ml levels of IAA, but still significantly higher than the control.

These data contrast with those of the previous experiment with *R. phaseoli* strain 442, where all levels of IAA suppressed growth and the 50 and 100 µg/ml levels completely suppressed growth. This growth response difference to the presence of IAA was considered to
Fig. 37 The response of *R. phaseoli*, strain QA 1062, grown in liquid culture at 4 concentrations of IAA.
- ○ = Control, ○ = 10 μg/ml IAA,
- △ = 50 μg/ml IAA, □ = 100 μg/ml IAA

Fig. 38 The effect of GA3 at 4 concentrations on the growth of *R. phaseoli*, strain QA 1062, grown in liquid culture.
- ○ = Control, ○ = 10 μg/ml GA3,
- △ = 50 μg/ml GA3, □ = 100 μg/ml GA3

Fig. 39 Growth response of *R. phaseoli*, strain QA 1062, grown in liquid culture at 4 concentrations of added kinetin.
- ○ = Control, ○ = 10 μg/ml Kin,
- △ = 50 μg/ml Kin, □ = 100 μg/ml Kin
be of possible significance in a plant root rhizosphere (with IAA present in root exudates) where competition exists between these two organisms for infection sites.

There was no growth response of R. phaseoli, strain QA 1062, to GA$_3$ (Fig. 38). A similar relationship was observed with R. phaseoli strain 442 (Fig. 26).

Incorporation of kinetin into the growth medium resulted in growth suppression of R. phaseoli, strain QA 1062, the suppression increasing with increasing concentration of kinetin (Fig. 39). At the 10 μg/ml level of kinetin, the growth of strain QA 1062, was slightly below, but not significantly lower than the control. The 50 and 100 μg/ml levels of kinetin significantly decreased the growth of strain QA 1062 below the control and 10 μg/ml addition throughout the experiment. The kinetin growth response data was similar to those shown earlier for R. phaseoli strain 442 (Fig. 27).

The growth curves of R. phaseoli, strain 1062, with a combination of growth regulators at the 10 μg/ml level are presented in Figs. 40, 41 and 42. There was essentially no growth differences, although all treatments with growth factors were slightly, but not significantly, lower than the control. The exception was the IAA/GA$_3$/Kinetin treatment which was significantly lower than the control, but not different from the other treatments.

The growth response differences of R. phaseoli, strain 1062, to combinations of growth regulators at the 50 μg/ml level (Figs. 43, 44 and 45) were greater and more distinguishable than those observed at the 10 μg/ml level (Figs. 40, 41 and 42). GA$_3$ in combination with
The effect of other growth regulators (GA₃ and kinetin) in combination with IAA (each growth regulator at 10 μg/ml) on growth of *R. phaseoli*, strain QA 1062, in liquid culture.

○ = Control, ○ = IAA/GA₃, △ = IAA/Kin,
□ = IAA/GA₃/Kin

**Fig. 40**

Growth response of *R. phaseoli*, strain QA 1062, to other growth regulators (IAA and kinetin) in combination with GA₃ (each growth regulator at 10 μg/ml).

○ = Control, ○ = IAA/GA₃, △ = GA₃/Kin,
□ = IAA/GA₃/Kin

**Fig. 41**

The effect of other growth regulators (IAA and GA₃) in combination with kinetin (each growth regulator at 10 μg/ml) on growth of *R. phaseoli*, strain QA 1062, in liquid culture.

○ = Control, ○ = IAA/Kin, △ = GA₃/Kin,
□ = IAA/GA₃/Kin

**Fig. 42**
Fig. 43

Growth response of *R. phaseoli*, strain QA 1062, (grown in liquid culture) to other growth regulators (GA3 and kinetin) in combination with IAA (each growth hormone at 50 μg/ml).

○ = Control, ○ = IAA/GA3, △ = IAA/Kin, □ = IAA/GA3/Kin

Fig. 44

The effect of other growth regulators (IAA and kinetin) in combination with GA3 (each growth regulator at 50 μg/ml) on growth of *R. phaseoli*, strain QA 1062, in liquid culture.

○ = Control, ○ = IAA/GA3, △ = GA3/Kin, □ = IAA/GA3/Kin

Fig. 45

The effect of other growth regulators (IAA and GA3) in combination with kinetin (each growth regulator at 50 μg/ml) on growth of *R. phaseoli*, strain QA 1062, in liquid culture.

○ = Control, ○ = IAA/Kin, △ = GA3/Kin, □ = IAA/GA3/Kin
IAA had little effect on the growth of strain QA 1062 (Fig. 31). However IAA/Kin and IAA/GA₃/Kin significantly reduced growth of strain QA 1062, presumably because of kinetin. These two treatments did not differ from each other.

The growth response of strain QA 1062 to combinations of growth regulators containing GA₃ (Fig. 44) was very similar to that of combinations with IAA (Fig. 43).

Growth of strain QA 1062 with all treatment combinations containing kinetin at the 50 μg/ml level (Fig. 45) were closely associated with each other and below the control. The Waller/Duncan test indicated, however, that this growth was not significantly lower than the control due to variation between duplicates within each treatment.

Growth of _R. phaseoli_, strain QA 1062, in liquid culture, with combinations of growth regulators at the 100 μg/ml level, are presented in Figs. 46, 47 and 48. Growth of strain 1062 in the control medium, without growth regulators, was significantly higher than all other treatment means throughout the experiment (Figs. 46, 47 and 48). The most predominant influence in these data is the dramatic kinetin inhibition, modified to a degree with GA₃, and intensified by IAA. IAA alone had previously been shown to have a moderate depressing effect on growth but together with kinetin the suppression of growth is greater than either growth regulator alone (Figs. 37 and 39). With the addition of GA₃ to IAA/Kin treatment, growth of strain QA 1062 occurred and was approximately equal to the growth observed within the IAA/GA₃ and GA₃/Kin treatments (Fig. 48).
The effects of other growth regulators (GA\textsubscript{3} and kinetin) in combination with IAA (at 100 μg/ml for each growth regulator) on growth of R. phaseoli, strain QA 1062, grown in liquid culture.

○ = Control, ○ = IAA/GA\textsubscript{3}, △ = IAA/Kin, □ = IAA/GA\textsubscript{3}/Kin

Growth response of R. phaseoli, strain QA 1062, (grown in liquid culture) to other growth regulators (IAA and kinetin) in combination with GA\textsubscript{3} (each growth hormone at 100 μg/ml).

○ = Control, ○ = IAA/GA\textsubscript{3}, △ = GA\textsubscript{3}/Kin, □ = IAA/GA\textsubscript{3}/Kin

The effect of other growth regulators (IAA and GA\textsubscript{3}) in combination with kinetin (at 100 μg/ml for each growth regulator) on growth of R. phaseoli, strain QA 1062, in liquid culture.

○ = Control, ○ = IAA/Kin, △ = GA\textsubscript{3}/Kin, □ = IAA/GA\textsubscript{3}/Kin
These data for *R. phaseoli*, strain QA 1062, when compared to those of *R. phaseoli*, strain 442, show unique differences between these two strains in their response to growth regulators, particularly IAA. *R. phaseoli*, strain 442, shows a definite sensitivity to the presence of increasing concentrations of IAA (Fig. 25) that cannot be modified by the presence of other growth regulators. On the other hand, *R. phaseoli*, strain QA 1062, shows the opposite response to IAA alone (Fig. 37). Furthermore, *R. phaseoli*, strain 442, gave almost a linear suppression of growth with increasing concentrations of kinetin (Fig. 13C) while strain QA 1062 showed an almost identical suppression of growth with 50 and 100 µg/ml treatments of kinetin (Fig. 39). Suppression of growth, however, was less than that shown for strain 442. The influence of IAA, in combination with other growth regulators at 50 µg/ml, on growth of strain 442 (Figs. 31, 32 and 33) was the same as 50 µg/ml of IAA alone (Fig. 25). At the 100 µg/ml concentration, combinations of IAA plus kinetin completely suppressed the growth of strain 442 indicating a possible synergism of IAA with Kin (Figs. 34, 35 and 36). With strain QA 1062, however, good growth was observed at 50 µg/ml for all combinations of growth regulators (Figs. 43, 44 and 45). At the 100 µg/ml concentration, the combination of IAA with kinetin was able to completely suppress growth of strain QA 1062 (Figs. 46 and 48) (similar to that observed for strain 442). The severity of the suppression was modified by the addition of GA₃.
5. *Rhizobium japonicum*, strain 110

*R. japonicum*, strain 110, showed little response to added IAA except for the 100 µg/ml level which suppressed growth through the 43rd hour (Fig. 49). After 43 hours, growth of strain 110 became equal to that of the control and the 10 µg/ml concentration of IAA. Growth with the 10 µg/ml rate remained approximately equal to the control throughout, while the 50 µg/ml rate of IAA increased growth of all treatments during the latter part of the growth curve. The Waller/Duncan test indicated that the 100 µg/ml rate of IAA resulted in significantly lower growth of strain 110 through the 83rd hour sampling. Growth of strain 110 with 50 µg/ml of IAA was significantly higher than all other treatments by the 91st hour and remained so through 115 hours. It is interesting to note that only one other organism showed a significant increase over the control with IAA present in the medium—*R. phaseoli* strain 1062 (Fig. 37).

The effect of various concentrations of GA$_3$ (0, 10, 50 and 100 µg/ml) on growth of *R. japonicum* strain 110 is presented in Fig. 50. There were no observed differences between rates of GA$_3$ on growth of strain 110, a finding observed for all other strains of *Rhizobium*.

Growth of strain 110 in the presence of kinetin was initially suppressed below that of the control medium for all rates (Fig. 51). Growth with the 10 and 100 µg/ml levels remained approximately equal throughout the experiment and were no different from the control from 87 hours to the end of the experiment. Growth of strain 110, was suppressed to the greatest extent by 50 µg/ml of kinetin and was significantly lower than the growth of strain 110 in the control throughout the experiment.
R. japonicum, strain 110, grown in liquid culture with 4 concentrations of IAA.

- Control, ○ = 10 ug/ml IAA,
- △ = 50 ug/ml IAA, □ = 100 ug/ml IAA

Fig. 50

R. japonicum, strain 110, grown in liquid culture with 4 concentrations of GA$_3$.

- Control, ○ = 10 ug/ml GA$_3$,
- △ = 50 ug/ml GA$_3$, □ = 100 ug/ml GA$_3$

Fig. 51

R. japonicum, strain 110, grown in liquid culture with 4 concentrations of kinetin.

- Control, ○ = 10 ug/ml Kin,
- △ = 50 ug/ml Kin, □ = 100 ug/ml Kin
The combination of other growth regulators, \( \text{GA}_3 \) and kinetin, with IAA and their effect on growth of \( R. \text{ japonicum} \) strain 110 at 10, 50 and 100 \( \mu \text{g/ml} \) are presented in Figs. 52, 53 and 54, respectively. Data for individual growth regulators was not included with the remainder of the growth curves for strain 110 because they were conducted prior to these experiments and therefore are not directly comparable. For comparison, refer to Figs. 49, 50 and 51. The combination of growth regulators at 10 \( \mu \text{g/ml} \) had little effect on growth of strain 110 (Fig. 52). During the initial growth of strain 110 (40 and 48 hours sampling times) all combinations of growth regulators suppressed growth significantly, while during the latter part of the experiment (87 hours onward), growth of the control was slightly, but significantly lower.

Growth regulators, at 50 \( \mu \text{g/ml} \) (Fig. 53), showed slightly greater growth responses but displayed the same trends in growth of strain 110 as observed for the 10 \( \mu \text{g/ml} \) level (Fig. 52). Growth of strain 110 within the IAA/Kin and IAA/\( \text{GA}_3 \)/Kin treatments showed the same growth responses relative to each other with growth slightly higher than that observed for the 10 \( \mu \text{g/ml} \) growth curves. The IAA/\( \text{GA}_3 \) combination stimulated growth of strain 110 to the greatest extent. At the end of the experiment (137 hours), the IAA/\( \text{GA}_3 \) treatment was no different from the other treatments, but all were significantly higher than the control. IAA alone, at 50 \( \mu \text{g/ml} \) (Fig. 49), gave the greatest positive growth response over the control during the last 40 hours of the experiment. This same relationship was observed for IAA in combination with \( \text{GA}_3 \) (Fig. 53) and was probably an IAA response.
Fig. 52 The effect of other growth regulators (GA3 and kinetin) in combination with IAA (all at 10 µg/ml) on growth of R. japonicum, strain 110, grown in liquid culture.

○ = Control, ○ = IAA/GA3, △ = IAA/Kin,
□ = IAA/GA3/Kin

Fig. 53 The response of R. japonicum, strain 110, to IAA in combination with GA3 and kinetin (all at 50 µg/ml) grown in liquid culture.

○ = Control, ○ = IAA/GA3, △ = IAA/Kin,
□ = IAA/GA3/Kin

Fig. 54 IAA in combination with GA3 and kinetin (at 100 µg/ml) and their effect on growth of R. japonicum, strain 110, grown in liquid culture.

○ = Control, ○ = IAA/GA3, △ = IAA/Kin,
□ = IAA/GA3/Kin
Combination of GA$_3$ and kinetin with IAA at 100 µg/ml (Fig. 54), resulted in greater growth responses by strain 110 than those shown previously in Figs. 52 and 53. The 100 µg/ml level of IAA in combination with kinetin suppressed growth of R. japonicum strain 110 severely, a response not noted previously with either IAA or kinetin alone (Fig. 49 and 51). The addition of GA$_3$ to the IAA/Kin combination significantly modified the growth suppression of IAA/Kin. IAA in combination with GA$_3$ provided a positive synergistic effect on growth of R. japonicum, strain 110. Contrast this response with that of 100 µg/ml IAA alone which gave the greatest suppression of growth during the early portion of the growth curve (Fig. 49).

The effect of GA$_3$ in combination with other growth regulators (IAA and Kin) at 3 concentrations on the growth of R. japonicum, strain 110, are presented in Figs. 35, 55, and 57 respectively. The relationship of growth for these growth regulators, to the control, is very similar to the growth curves for combinations of growth regulators with IAA at 10 µg/ml (Fig. 52) and not very indicative of any profound growth response.

When the concentration of each growth regulator was increased from 10 µg/ml to 50 µg/ml (Fig. 55) the differences in growth were magnified as observed previously (Fig. 53). Growth of strain 110 in the presence of GA$_3$/Kin combination was depressed significantly below all other treatments for the major portion of the experiment. Growth trends of the other treatments containing growth regulators were the same as previously observed (Fig. 53).

The growth of R. japonicum, strain 110, was influenced greatly by other growth regulators (IAA and kinetin) in combination with
Fig. 55  The effect of other growth regulators (IAA and kinetin) in combination with IAA (all at 10 μg/ml) on growth of *R. japonicum*, strain 110, grown in liquid culture.

○ = Control, ○ = IAA/GA₃, △ = GA₃/Kin,
□ = IAA/GA₃/Kin

Fig. 56  The response of *R. japonicum*, strain 110, to GA₃ in combination with IAA and kinetin (all at 50 μg/ml) grown in liquid culture.

○ = Control, ○ = IAA/GA₃, △ = GA₃/Kin,
□ = IAA/GA₃/Kin

Fig. 57  GA₃ in combination with IAA and kinetin (all at 100 μg/ml) and their effect on growth of *R. japonicum*, strain 110, grown in liquid culture.

○ = Control, ○ = IAA/GA₃, △ = GA₃/Kin,
□ = IAA/GA₃/Kin
GA$_3$ at 100 µg/ml (Fig. 57). The same growth relationships within the IAA/GA$_3$ and IAA/GA$_3$/Kin treatments, compared to the control, were the same as previously described for the treatment combinations with IAA at the 100 µg/ml level (Fig. 54). The GA$_3$/Kin treatment, which suppressed growth of strain 110 at the 50 µg/ml level (Fig. 56), showed the greatest suppression of growth of this organism from 63 hours to the end of the experiment when used at 100 µg/ml.

Growth response for combinations of growth regulators containing GA$_3$ (Figs. 55, 56 and 57) suggested two trends. First, the GA$_3$/IAA combination stimulates growth of $R$. japonicum strain 110, over the control, with increasing concentration of each. Second, growth of strain 110 in the presence of GA$_3$/Kin resulted in the greatest growth suppression below the control, increasing in magnitude with increasing concentration of each growth regulator.

Kinetin, in combination with IAA and/or GA$_3$ at 10 µg/ml and 50 µg/ml (Figs. 58 and 59), had little effect on growth of $R$. japonicum strain 110. When each growth regulator was used at 100 µg/ml (Fig. 60), growth, within all treatment combinations, was suppressed and significantly below the control. Treatments IAA/Kin and GA$_3$/Kin showed the greatest suppression of growth on strain 110 throughout the experiment. When all these growth regulators were present growth of strain 110 increased slightly towards the end of the experiment and was significantly higher than growth with IAA/Kin and GA$_3$/Kin.

6. *Rhizobium japonicum*, strain 123

$R$. japonicum, strain 123, was grown with varying concentrations of growth regulators in experiments analogous to those with $R$. japonicum, strain 110.
Fig. 58  The response of *R. japonicum*, strain 110, to kinetin in combination with IAA and GA₃ (all at 10 µg/ml) grown in liquid culture.

○ = Control,  ○ = IAA/Kin,
△ = GA₃/Kin,  □ = IAA/GA₃/Kin

Fig. 59  The effect, on growth, of *R. japonicum*, strain 110, grown in liquid culture with kinetin in combination with IAA and GA₃ (all at 50 µg/ml).

○ = Control,  ○ = IAA/Kin,
△ = GA₃/Kin,  □ = IAA/GA₃/Kin

Fig. 60  The effect on growth of *R. japonicum*, strain 110, grown in liquid culture with kinetin in combination with IAA and GA₃ (all at 100 µg/ml).

○ = Control,  ○ = IAA/Kin,
△ = GA₃/Kin,  □ = IAA/GA₃/Kin
japonicum, strain 110. These studies were done to determine whether these Rhizobium strains differed in their growth response to growth regulators.

The IAA completely suppressed the growth of strain 123, at the higher concentrations of 50 and 100 μg/ml (Fig. 61). Growth of strain 123 with 10 μg/ml of IAA did not differ from the control. These two sets of means differed significantly from the growth curves with 50 and 100 μg/ml IAA after 41 hours. This growth suppression differed markedly from the growth response of _R. japonicum_, strain 110, grown with IAA (Fig. 49). Growth of _R. japonicum_ strain 110 with 50 μg/ml IAA was greater than that observed in the control medium. The 100 μg/ml concentration of IAA resulted in growth equal to the control with no treatment rate giving complete inhibition.

The growth response of _R. japonicum_ strain 123 to GA$_3$ at various concentrations is presented in Fig. 62. As with the other organisms investigated thus far, there was little growth response to GA$_3$. The slight increase in growth of strain 123 with 100 μg/ml GA$_3$, after 60 hours, was significantly higher than the other treatments.

Increasing inhibition of growth of strain 123 was observed with increasing concentrations of kinetin (Fig. 63) although the inhibition was less than with IAA (Fig. 61). Growth of strain 123 in the control and 10 μg/ml level were identical throughout the experiment. The growth rate of strain 123, with 50 μg/ml kinetin after 48 hours, was significantly lower than the control and 10 μg/ml level of kinetin, but significantly higher than the rate of growth in the presence of 100 μg/ml kinetin.
Fig. 61  
*R. japonicum*, strain 123, grown in liquid culture in the presence of 4 concentrations of IAA.

- ○ = Control, ○ = 10 µg/ml IAA,
- △ = 50 µg/ml IAA,
- □ = 100 µg/ml IAA

Fig. 62  
*R. japonicum*, strain 123, grown in liquid culture with 4 concentrations of *GA*3.

- ○ = Control, ○ = 10 µg/ml GA3,
- △ = 50 µg/ml GA3,
- □ = 100 µg/ml GA3

Fig. 63  
*R. japonicum*, strain 123, grown in liquid culture in the presence of 4 concentrations of kinetin.

- ○ = Control, ○ = 10 µg/ml Kin,
- △ = 50 µg/ml Kin,
- □ = 100 µg/ml Kin
The growth response of *R. japonicum*, strain 123, to combinations of growth regulators containing IAA (Figs. 64, 65, and 66) or combinations containing \( \text{GA}_3 \) (Figs. 67, 68, and 69) are virtually identical. There was no difference in growth response of strain 123 to any combinations of growth regulators at the 10 \( \mu g/ml \) level (Figs. 64 and 67). Increasing the concentration of growth regulators to 50 \( \mu g/ml \) provided better separation of treatments (Figs. 65 and 68). IAA alone (Fig. 61), at 50 \( \mu g/ml \) (Fig. 61) was able to completely suppress growth of strain 123. Complete suppression of growth was not observed with \( \text{GA}_3 \) combinations (Figs. 65 and 68) indicating the ability of \( \text{GA}_3 \) to completely overcome IAA suppression. Kinetin also modified the suppressing effect of IAA and the growth of strain 123 was equal to that observed for strain 123 with kinetin alone (Fig. 61). When growth regulators were used at 100 \( \mu g/ml \) (Figs. 66 and 69), growth differences were magnified, but maintained the same relationship as observed at 50 \( \mu g/ml \). Growth of strain 123 with combination of IAA/\( \text{GA}_3 \) was equal to the control. The other treatments (containing growth regulators) were not different from each other, but were significantly lower than growth in the control medium and medium with IAA/\( \text{GA}_3 \).

The response of *R. japonicum*, strain 123, to combinations of growth regulators containing kinetin were negligible at the 10 \( \mu g/ml \) level (Fig. 70). Increasing the concentration of growth regulators to 50 \( \mu g/ml \) provided better separation of treatments, all of which were significantly below the control after 64 hours, but not different from each other (Fig. 71). The growth of strain 123, in the presence of combinations of growth regulators with kinetin at 50 \( \mu g/ml \),
The effect on growth of *R. japonicum*, strain 123, grown in liquid culture with other growth regulators (GA3 and kinetin) in combination with IAA (all growth regulators at 10 μg/ml),

○ = Control, △ = IAA/GA3, ○ = IAA/Kin, □ = IAA/GA3/Kin

Fig. 64

The growth response of *R. japonicum*, strain 123, grown in liquid culture in the presence of other growth regulators (GA3 and kinetin) in combination with IAA (all growth regulators at 50 μg/ml),

○ = Control, △ = IAA/GA3, ○ = IAA/Kin, □ = IAA/GA3/Kin

Fig. 65

The effect on growth of *R. japonicum*, strain 123, grown in liquid culture with other growth regulators (GA3 and kinetin) in combination with IAA (all growth regulators at 100 μg/ml),

○ = Control, △ = IAA/GA3, ○ = IAA/Kin, □ = IAA/GA3/Kin

Fig. 66
The growth response of *R. japonicum*, strain 123, grown in liquid culture with other growth regulators (IAA and kinetin) in combination with GA$_3$ (all growth regulators at 10 μg/ml).

〇 = Control, △ = IAA/GA$_3$, 〇 = GA$_3$/Kinetin, □ = IAA/GA$_3$/Kin

Growth of *R. japonicum*, strain 123, in liquid culture in the presence of other growth regulators (IAA and kinetin) in combination with GA$_3$ (all growth regulators at 50 μg/ml).

〇 = Control, △ = IAA/GA$_3$, 〇 = GA$_3$/Kinetin, □ = IAA/GA$_3$/Kin

The effect on growth of *R. japonicum*, strain 123, grown in liquid culture with other growth regulators (IAA and kinetin) in combination with GA$_3$ (all growth regulators at 100 μg/ml).

〇 = Control, △ = IAA/GA$_3$, 〇 = GA$_3$/Kinetin, □ = IAA/GA$_3$/Kin
approximates the growth curves observed for strain 123 grown with 50 μg/ml kinetin alone (Fig. 53). Growth response curves of strain 123 with 100 μg/ml of each growth regulator (Fig. 72), once again, shows the ability of kinetin to overcome the totally suppressing effect of IAA (Fig. 61). As before, the magnitude of suppression of growth, below the control, has increased. The severity of suppression of growth (for all treatments) approximates that observed for strain 123 grown with kinetin at 100 μg/ml (Fig. 63).

B. Growth of Rhizobium phaseoli in Sterile Soil

1. R. phaseoli strains 442 and QA 1062

Previous studies in liquid culture had shown unique growth responses to growth regulators alone, or in combination for R. phaseoli strains (442 and QA 1062). Of real concern was an evaluation of these responses in soil, since soil inorganic and organic colloids might be expected to alter their efficacy. Possible extrapolation of these data to actual soil-rhizosphere responses depends on such an evaluation. Concentrations of growth regulators used for these soil studies were 0, 10 and 100 μg/g dry soil for each growth regulator alone or in combination. Plate counts of R. phaseoli were made using soil-mannitol-yeast extract agar, to determine population changes for each treatment on days 0, 1, 2, 3 and 12 for strain 442, and days 0, 1, 2, 3 and 7 for strain QA 1062.

Population changes of R. phaseoli strains in soil amended with individual growth regulators (IAA, GA₃ and kinetin) at 0, 10 and 100 μg/g, are presented in Figs. 73, 74 and 75 for strain 442 and Figs. 76, 77 and 78 for strain QA 1062. R. phaseoli strains 442
Fig. 70 Growth of *R. japonicum*, strain 123, grown in liquid culture in the presence of other growth regulators (IAA and GA$_3$) in combination with kinetin (all growth regulators at 10 µg/ml).

○ = Control, △ = IAA/Kin, ○ = GA$_3$/Kin,

□ = IAA/GA$_3$/Kin

Fig. 71 The effect on growth of *R. japonicum*, strain 123, grown in liquid culture with other growth regulators (IAA and GA$_3$) in combination with kinetin (all growth regulators at 50 µg/ml).

○ = Control, △ = IAA/Kin, ○ = GA$_3$/Kin,

□ = IAA/GA$_3$/Kin

Fig. 72 The growth response of *R. japonicum*, strain 123, grown in liquid culture with other growth regulators (IAA and GA$_3$) in combination with kinetin (all growth regulators at 100 µg/ml).

○ = Control, △ = IAA/Kin, ○ = GA$_3$/Kin,

□ = IAA/GA$_3$/Kin
Fig. 73 Plate count (Days 0, 1, 2, 3 and 12) of R. phaseoli, strain 442, grown in sterile soil, with 3 concentrations of IAA.

- $\bigcirc$ = Control, $\square$ = 10 $\mu$g/g IAA, $\triangle$ = 100 $\mu$g/g IAA

Fig. 74 Plate count (Days 0, 1, 2, 3 and 12) of R. phaseoli, strain 442, grown in sterile soil, with 3 concentrations of $GA_3$.

- $\bigcirc$ = Control, $\square$ = 10 $\mu$g/g $GA_3$, $\triangle$ = 100 $\mu$g/g $GA_3$

Fig. 75 Plate count (Days 0, 1, 2, 3 and 12) of R. phaseoli, strain 442, grown in sterile soil, with 3 concentrations of kinetin.

- $\bigcirc$ = Control, $\square$ = 10 $\mu$g/g Kin, $\triangle$ = 100 $\mu$g/g Kin
Number of Organisms gram$^{-1}$ Dry Soil
and QA 1062 both multiplied at a faster rate with 10 µg/g IAA than in the control soil for the first two days (Figs. 73 and 76). The population then equalized and decreased slowly until the last sampling day. Numbers of both strains of *R. phaseoli* were decreased significantly by 100 µg/g IAA at day 1, after which strain QA 1062 recovered and by the second plating was approximately equal to the population of the control (Fig. 76). Strain 442 however, did not divide in the presence of 100 µg/g IAA and numbers remained significantly lower than all other treatments until the 12th day, where all treatment populations were equal. Similar growth responses of IAA at 100 µg/ml were observed in the liquid culture experiment for both strains (Figs. 25 and 37).

Increasing concentrations of GA₃ had little effect on numbers of either strain of *R. phaseoli* grown in sterile soil (Figs. 74 and 77). Populations within both GA₃ concentrations (10 and 100 µg/g), for both strains, were slightly above the control throughout the experiment. Again, the response of strain 442 and QA 1062, in liquid culture (Figs. 26 and 38 respectively) were very similar to that found in sterile soil.

Populations of both strains of *R. phaseoli* growing in sterile soil with 10 µg/g kinetin increased slightly, but significantly, above the control for the first two days only (Figs. 75 and 78). Increasing the kinetin concentration to 100 µg/g did not greatly affect either strain of *R. phaseoli* for the first two days after which the population of strain 442 remained significantly below that of the control and the treatment with 10 µg/g kinetin. Strain QA 1062,
Fig. 75 Plate count (Days 0, 1, 2, 3 and 7) of R. phaseoli, strain QA 1062, grown in sterile soil, with 3 concentrations of IAA.

○ = Control, ○ = 10 µg/g IAA,
△ = 100 µg/g IAA

Fig. 77 Plate count (Days 0, 1, 2, 3 and 7) of R. phaseoli, strain QA 1062, grown in sterile soil, with 3 concentrations of GA₃.

○ = Control, ○ = 10 µg/g GA₃,
△ = 100 µg/g IAA

Fig. 78 Plate count (Days 0, 1, 2, 3 and 7) of R. phaseoli, strain QA 1062, grown in sterile soil with 3 concentrations of kinetin.

○ = Control, ○ = 10 µg/g Kin,
△ = 100 µg/g Kin
however, showed no significant effect until the last sampling day (day 7). Greater growth suppression with 100 µg/ml kinetin in liquid culture had occurred for strain 442 than for strain QA 1062. This suppression of growth, for both strains of \textit{R. phaseoli} was also evident in the sterile soil experiment, but the magnitude of suppression was less than in liquid culture. Considering the structure of kinetin with a pKa at 2.7 and 9.9, there is a high probability of this molecule being actively adsorbed on soil exchange sites, thus reducing the effective concentration available to effect microbial growth.

The effect of mixtures of growth regulators on numbers of \textit{R. phaseoli} in sterile soil are presented in Figs. 79, 80, 31 and 82 for strain 442 and Figs. 83, 34, 85 and 35 for strain QA 1062. The combination of IAA/GA$_3$, both at 10 µg/g had no effect on strain 442 (Fig. 79) and QA 1062 (Fig. 83). Increasing the concentration of each growth regulator to 100 µg/g, however, significantly suppressed cell division of strain 442 throughout the experiment (Fig. 79). Growth of strain QA 1062 was suppressed for one day after which cell division occurred and the final population was not different from the other treatments (Fig. 33). The plate count data at 100 µg/g IAA for strain 442 (Fig. 73) and strain QA 1062 (Fig. 76), was very similar to that observed for 100 µg/g of IAA/GA$_3$ for both strains. The response, therefore, was attributed to the IAA portion of the IAA/GA$_3$ combination. Growth curves for strain 442, in liquid culture, at 100 µg/ml of IAA/GA$_3$ (Fig. 34) and strain QA 1062 (Fig. 46) showed the same trends but with a greater degree of
Fig. 79  Plate count (Days 0, 1, 2, 3 and 12) of R. phaseoli, strain 442, grown in sterile soil with 3 concentrations of IAA/GA$_3$.

○ = Control,  □ = 10 µg/g IAA/GA$_3$,  
Δ = 100 µg/g IAA/GA$_3$

Fig. 80  Plate count (Days 0, 1, 2, 3 and 12) of R. phaseoli, strain 442, grown in sterile soil with 3 concentrations of IAA/Kin.

○ = Control,  ○ = 10 µg/g IAA/Kin,  
Δ = 100 µg/g IAA/Kin

Fig. 81  Plate count (Days 0, 1, 2, 3 and 12) of R. phaseoli, strain 442, grown in sterile soil with 3 concentrations of GA$_3$/Kin.

○ = Control,  □ = 10 µg/g GA$_3$/Kin,  
Δ = 100 µg/g GA$_3$/Kin

Fig. 82  Plate count (Days 0, 1, 2, 3 and 12) of R. phaseoli, strain 442, grown in sterile soil with 3 concentrations of IAA/GA$_3$/Kin.

○ = Control,  ○ = 10 µg/g IAA/GA$_3$/Kin,  
Δ = 100 µg/g IAA/GA$_3$/Kin
Log Number of Organisms gram$^{-1}$ Dry Soil

Fig. 79

Time (Days)

0 1 2 3 12
Fig. S3 Plate count (Days 0, 1, 2, 3 and 7) of R. phaseoli, strain QA 1062, grown in sterile soil with 3 concentrations of IAA/GA<sub>3</sub>.

○ = Control, ○ = 10 µg/g IAA/GA<sub>3</sub>,
△ = 100 µg/g IAA/GA<sub>3</sub>

Fig. 84 Plate count (Days 0, 1, 2, 3 and 7) of R. phaseoli, strain QA 1062, grown in sterile soil with 3 concentrations of IAA/Kin.

○ = Control, ○ = 10 µg/g IAA/Kin,
△ = 100 µg/g IAA/Kin

Fig. 85 Plate count (Days 0, 1, 2, 3 and 7) of R. phaseoli, strain QA 1062, grown in sterile soil with 3 concentrations of GA<sub>3</sub>/Kin.

○ = Control, ○ = 10 µg/g GA<sub>3</sub>/Kin,
△ = 100 µg/g GA<sub>3</sub>/Kin

Fig. 86 Plate count (Days 0, 1, 2, 3 and 7) of R. phaseoli, strain QA 1062, grown in sterile soil with 3 concentrations of IAA/GA<sub>3</sub>/Kin.

○ = Control, ○ = 10 µg/g IAA/GA<sub>3</sub>/Kin,
△ = 100 µg/g IAA/GA<sub>3</sub>/Kin
Log Number of Organisms gram$^{-1}$ Dry Soil

Time (Days)
suppression of growth in liquid culture than that observed in sterile soil. The slight stimulation of growth for both strains of *R. phaseoli*, in soil, at the 10 μg/g concentration of IAA/GA$_3$ was not observed in the liquid culture data (Figs. 28 and 40) and could be a consequence of effective concentrations being less than 10 μg/g because of soil interactions.

IAA, in combination with kinetin, at 10 μg/g soil, had little effect on numbers of *R. phaseoli*, strain 442, (Fig. 80) and strain QA 1062 (Fig. 84). This response was similar to that found in the liquid culture experiment for strain 442 (Fig. 28) and strain QA 1062 (Fig. 40). The addition of IAA/Kin to soil at 100 μg/g significantly suppressed the population of both strains of *R. phaseoli* below the control throughout the experiment. Strain QA 1062 (Fig. 84) initially decreased in population (1 day) after which growth increased slightly but was still significantly below the control. This same pattern was observed for strain QA 1062 grown with 100 μg/g IAA alone (Fig. 76), but the magnitude of the suppression of cell division, was greater with the IAA/Kin combination. Growth of strain QA 1062, in liquid culture with 100 μg/ml IAA/Kin, was completely suppressed (Fig. 46), but this was not observed in the sterile soil experiment (Fig. 84). As previously mentioned, this could be a consequence of a reduction in effective concentration of growth regulators because of interaction with soil. It was noted that the growth of strain QA 1062 in liquid medium with 50 μg/ml of IAA/Kin (Fig. 43), more closely approximates that of strain QA 1062 grown with 100 μg/g IAA/Kin in sterile soil (Fig. 84).

The population of strain 442, grown with 100 μg/g
IAA/Kin (Fig. 30) actually continued to decline throughout the experiment. This population decline was not observed in the 100 μg/g IAA treatment (Fig. 74) where the population was relatively constant throughout the experiment. This not only indicated a decline in numbers of strain 442 in the presence of IAA/Kin at 100 μg/g but a possible bacteriocidal effect of this combination on strain 442, as well.

The combination of GA₃/Kin had little effect on growth of R. phaseoli strain QA 1062 grown in sterile soil (Fig. 85). By the 7th day, however, the population of strain QA 1062 in soil with 10 μg/g GA₃/Kin was slightly but significantly less than the control, but significantly higher than with 100 μg/g GA₃/Kin. The growth of strain QA 1062 in liquid culture with 10 μg/ml GA₃/Kin (Fig. 41) correlates closely with growth of strain QA 1062 with the same treatment and concentration of growth regulators in sterile soil (Fig. 85). At 100 μg/ml of GA₃/Kin, greater suppression occurred in the liquid culture experiment (Fig. 47) than was observed in sterile soil (Fig. 85).

Strain 442 with GA₃ in combination with kinetin at 10 μg/g soil increased slightly in numbers above the control. Very little difference in growth between the control and GA₃/Kin treatment at 10 μg/ml had been shown in liquid culture (Fig. 29). Increasing the concentration of GA₃/Kin to 100 μg/g in sterile soil significantly decreased the population of strain 442 below all other treatments during the last two sampling dates (days 3 and 12) (Fig. 81). This response approximates that of strain 442 grown in the presence of
kinetin at 100 µg/g (Fig. 75) except for the lack of initial 1 day suppression of numbers which could possibly be attributed to the ameliorating effect of GA₃. Growth of strain 442 in liquid culture with GA₃/Kin at 100 µg/ml (Fig. 34) showed a greater suppression of growth than the comparable treatment in sterile soil (Fig. 81).

The combination of all growth regulators (IAA/GA₃/Kin) at the 10 µg/g level did not greatly effect the population of R. phaseoli strain 442 (Fig. 32) and QA 1062 (Fig. 36) throughout the experiment. The plate count estimates of strain QA 1062 (Fig. 36), after one day at the 10 µg/g level were significantly lower than all other treatments. Considering the previous growth trends of strain QA 1062 with growth regulators alone or in combination at the 10 µg/g level, the validity of this particular data point is highly questionable. Growth in liquid culture for both strain 442 (Fig. 28) and strain QA 1062 (Fig. 40), at 10 µg/ml of IAA/GA₃/Kin, was consistently and significantly lower than the control with no indication of recovery. Strain 442 was suppressed to a greater degree than strain QA 1062. The population of strain QA 1062 in sterile soil was unaffected by 100 µg/g IAA/GA₃/Kin for the first 3 days after which the population of strain QA 1062 was significantly lower than the other treatments (Fig. 35). Strain 442, however, showed a significant decline in population in the presence of IAA/GA₃ Kin at 100 µg/g which was similar to that with 100 µg/g IAA/Kin (Fig. 84). Growth of strain 442 was completely suppressed with 50 and 100 µg/ml IAA/GA₃/Kin (Figs. 31 and 34) in liquid culture. Growth of strain QA 1062 in liquid culture with 100 µg/ml IAA/GA₃/Kin
(Fig. 46) was significantly lower than the control, but this was not the case in the sterile soil study. This again indicates 1.) the ability of the soil to reduce the effective concentration of growth regulators and 2.) the possibility that strain 442 is more sensitive to the presence of growth regulators than strain QA 1062.

2. Response of *R. phaseoli* strains 442 and QA 1062 to Growth Regulators When Both Are Added to Sterile Soil

Unique differences in response to growth regulators, alone or in combination, were noted for each individual strain of *R. phaseoli* grown in sterile soil or in liquid culture. Since, both strains of *R. phaseoli* (442 and QA 1062) applied to soil would be in direct competition for the same nodule site on the roots of *P. vulgaris*, it seemed important to determine if a differential response to growth regulators would give a competitive advantage to one strain over the other when grown together. Tolerance, stimulation or inhibition of either strain of *R. phaseoli* by growth regulators within the plant rhizosphere and in particular the site of nodule formation could influence which strain of *R. phaseoli* predominates.

*R. phaseoli* strains 442 and QA 1062 could be distinguished by differences in colony morphology as previously described in the Material and Methods section. This procedure was useful in studies where both cultures were added as a mixture to sterile soil.

Population changes of *R. phaseoli* (strains 442 and QA 1062), grown in sterile soil with 3 levels (0, 10 and 100 μg/g) of IAA, GA₃, and kinetin are presented in Figs. 37, 38, and 39 respectively. The population trends for the control and 10 μg/g rate of IAA of
Plate count (Days 0, 1, 2, 3 and 7) of the mixture of R. phaseoli strains 442 and QA 1062, grown in sterile soil, with 3 concentrations of IAA.
For strain 442: ○ = Control, ○ = 10 μg/g IAA
△ = 100 μg/g IAA
For strain QA 1062: ◇ = Control, ◇ = 10 μg/g IAA
△ = 100 μg/g IAA

Plate count (Days 0, 1, 2, 3 and 7) of the mixture of R. phaseoli strains 442 and QA 1062, grown in sterile soil, with 3 concentrations of GA₃.
For strain 442: ○ = Control, ○ = 10 μg/g GA₃
△ = 100 μg/g GA₃
For strain QA 1062: ◇ = Control, ◇ = 10 μg/g GA₃
△ = 100 μg/g GA₃

Plate count (Days 0, 1, 2, 3 and 7) of the mixture of R. phaseoli strains 442 and QA 1062, grown in sterile soil, with 3 concentrations of kinetin.
For strain 442: ○ = Control, ○ = 10 μg/g Kin
△ = 100 μg/g Kin
For strain QA 1062: ◇ = Control, ◇ = 10 μg/g Kin
△ = 100 μg/g Kin
Log Number of Organisms Gram$^{-1}$ Dry Soil
the combined strain data (Fig. 37), approximates that for each individual strain of *R. phaseoli* (Fig. 73 for strain 442 and Fig. 76 for strain QA 1062). Initial suppression of the population of strain 442 by 100 μg/g IAA was not as severe in this study when compared to data where strain 442 was grown alone (Fig. 73). However, the final population was significantly lower than the control and 10 μg/g IAA amendment, for both strains, for the entire experiment. In the previous study, strain QA 1062, when grown alone (Fig. 76), was significantly suppressed by the 100 μg/g IAA amendment only on day one, after which the population increased and was no different than the control. When strain QA 1062 was grown in the presence of strain 442 with 100 μg/g IAA, the population declined more rapidly than the control treatment (Fig. 37).

There were several trends that should be noted in the combined strain data with IAA. First was that the population of strain QA 1062 grown with IAA or even the control soil, were higher, and in most cases significantly higher, than the population of strain 442. Second, the population of strain QA 1062, with the 100 μg/g level of IAA, by day 7, was no different than the population found in the control and 10 μg/g rate for strain 442 but still significantly lower than the population noted for the control and 10 μg/g concentration of IAA for strain QA 1062.

A slight, but non-significant stimulation of both strain 442 and QA 1062, above the control, had been noted when these strains were grown separately, in sterile soil, amended with GA₃ (Fig. 74 for strain 442 and Fig. 77 for strain QA 1062). This same trend
was evident when these strains were combined (Fig. 86) except for the highest level of GA$_3$ on day 7, where the population of strain QA 1062 was significantly lower than the final population for the other treatments. More variation for treatment response was noted for strain 442 in combination (Fig. 88) as compared to when strain 442 was grown alone (Fig. 74). All GA$_3$ treatments reduced the numbers of strain 442 below the control, and some of these differences were significant (Fig. 88).

Kinetin did not stimulate cell division of either strain when they were combined in soil (Fig. 89), as was previously observed for strain 442 (Fig. 75) and strain QA 1062 (Fig. 78) alone. In the combined strain study (Fig. 89) strain 442 was sensitive to all levels of kinetin, with both 10 and 100 µg/g kinetin generally decreasing cell numbers of strain 442 below the control population until the last sampling (day 7). At day 7 the population in all treatments were significantly lower than with strain QA 1062.

There was little difference in numbers of _R. phaseoli_ in the control soil and soil with 10 µg/g treatment combinations of growth regulators, for either strain, through the first two days (Figs. 90, 91, 92 and 93). By day 2 the maximum cell numbers were achieved after which the populations with treatments of strain 442 declined at a greater rate than for strain QA 1062. Treatment combinations with the 100 µg/g level of growth regulators containing IAA reduced cell division for both strains, and numbers were approximately equal through the first 3 days (Figs. 90, 91 and 93). After this time strain 442 declined and became significantly lower
Fig. 90 Plate count (Days 0, 1, 2, 3 and 7) of the mixture of R. phaseoli, strains 442 and QA 1062, grown in sterile soil, with 3 concentrations of IAA/GA$_3$.

For strain 442: $\bigcirc$ = Control, $\bullet$ = 10 $\mu$g/g IAA/GA$_3$, $\triangle$ = 100 $\mu$g/g IAA/GA$_3$

For strain QA 1062: $\bigcirc$ = Control, $\bullet$ = 10 $\mu$g/g IAA/GA$_3$, $\square$ = 100 $\mu$g/g IAA/GA$_3$

Fig. 91 Plate count (Days 0, 1, 2, 3 and 7) of the mixture of R. phaseoli, strains 442 and QA 1062, grown in sterile soil, with 3 concentrations of IAA/Kin.

For strain 442: $\bigcirc$ = Control, $\bullet$ = 10 $\mu$g/g IAA/Kin, $\triangle$ = 100 $\mu$g/g IAA/Kin

For strain QA 1062: $\bigcirc$ = Control, $\bullet$ = 10 $\mu$g/g IAA/Kin, $\square$ = 100 $\mu$g/g IAA/Kin

Fig. 92 Plate count (Days 0, 1, 2, 3 and 7) of the mixture of R. phaseoli, strains 442 and QA 1062, grown in sterile soil, with 3 concentrations of GA$_3$/Kin.

For strain 442: $\bigcirc$ = Control, $\bullet$ = 10 $\mu$g/g GA$_3$/Kin, $\triangle$ = 100 $\mu$g/g GA$_3$/Kin

For strain QA 1062: $\bigcirc$ = Control, $\bullet$ = 10 $\mu$g/g GA$_3$/Kin, $\square$ = 100 $\mu$g/g GA$_3$/Kin

Fig. 93 Plate count (Days 0, 1, 2, 3 and 7) of the mixture of R. phaseoli, strains 442 and QA 1062, grown in sterile soil with 3 concentrations of IAA/GA$_3$/Kin.

For strain 442: $\bigcirc$ = Control, $\bullet$ = 10 $\mu$g/g IAA/GA$_3$/Kin, $\triangle$ = 100 $\mu$g/g IAA/GA$_3$/Kin

For strain QA 1062: $\bigcirc$ = Control, $\bullet$ = 10 $\mu$g/g IAA/GA$_3$/Kin, $\square$ = 100 $\mu$g/g IAA/GA$_3$/Kin
Log Number of Organisms gram$^{-1}$ Dry Soil
than all other treatments. This pattern was consistent with that observed for both strains of *R. phaseoli* when previously grown with 100 μg/g IAA (Fig. 87), and demonstrates the differential inhibitory effect of IAA. An exception to this pattern was noted for strain QA 1062 at day 2 when grown with 100 μg/g IAA/Kin (Fig. 91). Here the population increased significantly and doubt exists as to the validity of this data point.

The GA3/Kin combination in sterile soil with combination of strains (Fig. 92) showed little difference in the population trends as compared to the individual strain data (Fig. 81) for strain 442 and Fig. 85 for strain QA 1062).

3. Competition Studies of *R. phaseoli* and *R. japonicum* Strains

Previous studies in liquid culture, and sterile soil, indicated a similarity in response of the *R. phaseoli* and *R. japonicum* strains to IAA alone or in combination with other growth regulators. Growth of *R. phaseoli*, strain 442, in liquid culture, showed increased suppression with increasing concentrations of IAA (Fig. 25) as did *R. japonicum*, strain 123, (Fig. 61). *R. phaseoli*, strain QA 1062, (Fig. 37) and *R. japonicum*, strain 110, (Fig. 49), however, showed only initial suppression of growth after which stimulation, above the control, occurred.

This similarity of growth response led to the hypothesis that tolerance, and/or stimulation in the presence of IAA of *R. phaseoli*, strain QA 1062 and *R. japonicum*, strain 110 might provide a competitive advantage over *R. phaseoli*, strain 442, and *R. japonicum*, strain 123 for nodule sites in their respective host root environment.
An experiment was conducted in the growth chamber to determine if this indeed was the case. Seeds of *P. vulgaris* were placed in Leonard jars containing sterile vermiculite and inoculated with either strain of *R. phaseoli* alone or in an equal mixture. The Leonard jars were placed in an environmental chamber and watered as previously described in the Materials and Methods section. At the end of the experiment the plants were harvested and 25 nodules from each individual strain, and 60 nodules from the combined strain treatment were randomly selected and used in the serological determination of the infecting strain.

The results of the serological reactions are presented in Table 2. The serology on the 25 nodules taken from *P. vulgaris* grown in association with *R. phaseoli*, strain QA 1062, showed a good agglutination reaction with nodule extracts as well as the original parent strain. Sixty percent (15 out of 25) of the nodules shows a positive agglutination reaction while the remaining nodules gave no reaction. This negative reaction was attributed to inadequate turbidity of cells from the extremely small nodules formed on the root of *P. vulgaris*.

No agglutination reaction occurred from the *R. phaseoli*, strain 442 nodules nor from the original parent culture. *P. vulgaris* plants were healthy and showed no signs of nitrogen deficiency indicating that the organism present was capable of forming effective nodules. It was therefore concluded that the strain of 442 used in this experiment had either mutated from the original strain used to produce the antiserum or the antiserum had deteriorated and was unuseable. The latter is most logical.
Table 2. Serological reaction of nodules from *P. vulgaris* grown in association with *R. phaseoli* strains 442 and QA 1062 alone or in combination.

<table>
<thead>
<tr>
<th>R. PHASEOLI STRAIN</th>
<th># NODULES TESTED</th>
<th># OF POSITIVE REACTIONS</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>442</td>
<td>1062</td>
</tr>
<tr>
<td>442</td>
<td>25</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>1062</td>
<td>25</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>442 + 1062</td>
<td>60</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

* No reaction with antisera for strain 442 nor the parent strain. Plants showed no sign of nitrogen deficiency and it was therefore assumed that 100% of the nodules were formed by strain 442.

** Nodules from *P. vulgaris* were extremely small and it is sometimes difficult to attain adequate turbidity for agglutination to occur.
The serology on the combined strain nodules indicated that 100% of the nodules sampled were formed by *R. phaseoli*, strain QA 1062. This strain completely predominated over strain 442 in competition for nodule sites.

Work had previously been done in this laboratory (Vidor, 1977) on the relative competitiveness for nodule sites between strains of *R. japonicum*. In this work, *G. max* var. Amsoy-71, grown in sand-nutrient solution in Leonard jars, was inoculated with strains of *R. japonicum* (strains 110, 123, 532C and 586) alone or in a mixture. Serological reaction of the nodules indicated that each strain, applied individually, produced 100% of the nodules. When the strains were applied as a mixture, 78% of the nodules formed were in serogroup 110 with 5% of the nodules formed by strain 123.

These data supported the original hypothesis and showed a positive correlation between tolerance of IAA and competiveness between strains for nodule sites.
DISCUSSION

The increased microbial population associated with plant root systems as compared to that found in the soil has been attributed to the organic nutrients supplied by plant root exudates (Hale et al., 1971; Rovira, 1971; Vancura, 1964). The analysis of plant root exudates, as found in the literature, mainly reflects the amino acid distribution, sugar composition, organic acid content of the cell sap, etc. with little indication of the presence, or the effects, of plant growth regulators on the rhizosphere microbial population.

In this study, investigations of the possible stimulatory, inhibitory or neutral effect of plant growth regulators (IAA, GA$_3$ and kinetin) on free living, associative and symbiotic dinitrogen fixing organisms were conducted. Later, plant growth regulators were also considered as factors potentially capable of conferring a competitive advantage of one Rhizobium strain over another when in competition for nodule sites.

The initial experiments in liquid culture with A. beijerinckii, A. brasilense, R. phaseoli strain 442 and QA 1062, and R. japonicum strains 110 and 123 indicated some very unique and interesting differences in response to various combination and concentrations of plant growth regulators (Table 3). It was found that GA$_3$ had little effect on growth of these organisms with the exception of A. beijerinckii which was stimulated by the presence of GA$_3$ (Fig 1) and a synergistic stimulation of R. japonicum by GA$_3$ in combination
Table 3. Summary of effects on growth of free-living associative and symbiotic dinitrogen fixing organisms grown in liquid culture with various concentration and combinations of growth regulators. (The degree of response is relative to the control).

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>IAA</th>
<th>GA3</th>
<th>Kin</th>
<th>IAA/GA3</th>
<th>IAA/Kin</th>
<th>GA3/Kin</th>
<th>IAA/GA3/Kin</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. beijerinckii</td>
<td>10</td>
<td>50</td>
<td>100</td>
<td>10</td>
<td>50</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>A. brasilense*</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>R. phaseoli (442)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(QA)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>R. phaseoli 1062</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(110)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. japonicum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. japonicum (123)</td>
<td></td>
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</tr>
</tbody>
</table>

Concentrations used for A. brasilense were 1.0, 10.0 and 50.0 μg/ml for each growth regulator. The 1.0 μg/ml rate of growth regulators was not included in this table. The growth response for this concentration approximates that of the control and/or the 10 μg/ml rate response.

Key: Suppression - □ = Slight, □ = Moderate □ = Extreme
Stimulation - □ = Slight, □ = Moderate □ = Extreme
No effect □
with IAA (Fig. 55-57). The two growth regulators that consistently either stimulated or inhibited growth of these organisms were IAA and kinetin alone or in combination with each other.

One particularly exciting aspect of these studies was the differential in response to IAA and kinetin between strains of *Rhizobium* which strongly suggests a possible explanation for differences in competitiveness. It was proposed that stimulation and/or tolerance to IAA and kinetin by *R. phaseoli*, strain QA 1062 (Fig. 37 and Fig. 39, respectively) and *R. japonicum*, strain 110 (Fig. 49 and Fig. 51, respectively) could possibly provide a competitive advantage for host nodule sites over *R. phaseoli* strain 442 (Fig. 25 and Fig. 27, respectively) and *R. japonicum* strain 123 (Fig. 61 and Fig. 63) which were severely inhibited by IAA and kinetin. In competitive studies using *R. phaseoli* strain 442 and QA 1062 (Table 2) strain QA 1062 completely predominated over strain 442 and formed 100 percent of the nodules. Previous work in this laboratory (Vidor, 1977) on competitiveness of *R. japonicum* strains indicated that strain 110, in a mixed culture, formed 78% of the nodules while strain 123 formed only 5%. These data support the hypothesis as previously stated and provide a potential method of screening *Rhizobium* strains for competitiveness.

How might IAA and kinetin confer a competitive advantage to one *Rhizobium* strain over another? The possible hypothesis proposed must remain just a hypothesis for the time being. First, observations of the liquid culture experiments showed that large amounts of extracellular polysaccharides were produced in response to the presence
of growth regulators. This was particularly true for the two R. japonicum strains. Capsular polysaccharides have been identified as lectin receptors on rhizobial cells and as being important to nodulation in legumes. Differential stimulation of polysaccharide production by growth regulators could explain the observed results and increase the affinity for the Rhizobium cell surface.

A second consideration is that growth regulators could selectively stimulate an increase in cell numbers of one strain over another in the vicinity of the root. Nutman (1965) indicated that fewer than 100 Rhizobium cells were required, in the rhizosphere, to form the first nodule after which progressively larger numbers of Rhizobium were required to ensure continued nodulation. The combined strain data for R. phaseoli grown in sterile soil (Figs. 87 through 93) as well as the liquid culture data (Figs. 25 through 48) initially showed no population differences after which the population of strain 442 dropped off much more rapidly than strain QA 1062. Strain QA 1062, in sterile soil, usually had a significantly higher cell number at the end of the experiment than strain 442. The ability to maintain a large number of cells in the rhizosphere over a longer period of time appears to be of importance in determining which strain of Rhizobium predominates in competition for nodule sites.

The question arises - can these particular growth regulators (IAA and kinetin) appear in the root rhizosphere in sufficient quantities to exert an influence on the growth of the rhizosphere microorganisms such as the associative or dinitrogen fixing bacteria? The possibility of attaining a concentration of 100 μg/ml of any of these growth regulators in the rhizosphere seems doubtful.
The exact concentration, at microsites on the root surface, however, are suspected to be higher than the literature now indicates (approximately 10 μg/l cytokinin, 1.5 μg/l IAA, (Puppo and Rigaud, 1977). Higher concentrations could be attained due to minimal dilution at the root surface and the contribution of microbially produced plant-like growth regulators to the growth regulator pool at the root surface. From the success of the competition studies reported in this dissertation, it is inferred that adequate concentrations of growth regulators are available to effect growth of the associated rhizosphere microbial populations.

The free living and associative dinitrogen fixing organisms used in this study showed opposite effects to the presence of growth regulators. IAA had no effect on growth of *A. beijerinckii* (Fig. 1) while GA₃ stimulated (Fig. 2) and kinetin suppressed growth (Fig. 3). On the other hand growth of *A. brasilense* was not affected by the presence of GA₃ (Fig. 14), stimulated by kinetin (Fig. 15) and suppressed by IAA (Fig. 13). These responses to growth regulators could well determine if an active association could develop in the plant rhizosphere. Determination of the type and quantity of growth regulators released by the plant could predict whether an association could occur and possibly explain why previous attempts at establishing these organisms, particularly *Azotobacter* species, have either succeeded or failed (Brown et al., 1962; Clark, 1948; Vancuna et al., 1959; Timonin, 1948).
The persistence, in an active form, of plant and microbially produced IAA found in the root rhizosphere is also significant in evaluating their efficacy. Persistence appears to be dependent on two factors. The first is the presence of IAA-oxidase, or peroxidase, which rapidly oxidizes IAA to an inactive form. IAA-oxidase has been shown to be compartmentalized in membranes of root cells. As long as the root cells remain intact the IAA-oxidase regulates IAA within the cell and appears to have minimal effect on rhizosphere IAA oxidation. The literature shows little indication that bacterial cells have the ability to oxidize IAA utilizing peroxidase. The second factor concerns the presence and type of phenols available in the root exudates or surrounding soil solution. Monohydroxyphenols such as paracoumaric and parahydroxybenzoic acids promote oxidation of IAA by IAA oxidase while dihydroxyphenols such as caffeic acid have the opposite effect and tends to protect IAA from oxidation (Thimann, 1977). The persistence of these IAA-phenol molecules is unknown. There was little indication in the literature that IAA can be extracted from soil.

The fate of kinetin in the soil is assumed to parallel that of other purine bases in that they can be metabolized by a variety of soil organisms. Purines as well as pyrimidines and nucleic acids are readily adsorbed, however, by clays and may be protected to a significant extent from microbial degradation. This adsorption could well explain why purine based cytokinins persist in soil and were able to be extracted by Van Staden and Dimalla (1976) from soils supporting plant species having a symbiotic relationship with microorganisms.
CONCLUSIONS

1. Associative and free living dinitrogen fixing bacteria responded differentially in liquid culture to the presence of plant growth regulators (IAA, GA_3 and kinetin) alone or in combination, at concentrations ranging from 0 to 100 μg/ml.

2. *A. beijerinckii*, grown in liquid culture, showed increased growth in the presence of GA_3, a suppression of growth with increasing concentrations of kinetin and no response to IAA. Growth of *A. brasilense* was suppressed by IAA, not affected by GA_3 but increasing concentrations of kinetin showed a corresponding increase in growth opposite to that of *A. beijerinckii*.

3. Stimulation of growth, in liquid culture, of *R. phaseoli*, strain QA 1062 and *R. japonicum*, strain 110, was observed with IAA and a slight suppression of growth in the presence of kinetin. *R. phaseoli*, strain 442 and *R. japonicum*, strain 123, were severely suppressed in growth with IAA and moderately suppressed by kinetin.

4. Sterile soil growth studies using *R. phaseoli* strains 442 and QA 1062 showed the same general growth responses to the presence of growth regulators as observed in liquid culture, the magnitude of which was somewhat less. This reduction in response was undoubtedly caused by adsorption or complexation of growth regulators with the soil which would effectively reduce the active concentration.

5. Competitiveness studies between strains of *R. phaseoli* and strains of *R. japonicum* confirmed a competitive advantage to those strains that were stimulated or most tolerant to IAA and kinetin.
APPENDIX A

Soil Extract-Mannitol-Yeast Extract Media

Yeast Extract 1.0 g  
Soil Extract* 200 ml  
Mannitol 10 g  
Distilled water 800 ml  
Adjust to pH 7.2

If solid media is desired add 15 g Agar

* To 1000 g fertile soil add 1000 ml tap water and autoclave (121° C) 20 minutes. Add approximately 0.5 g CaCO₃ to flocculate colloidal material and filter to clarify.
APPENDIX B

Azospirillum Medium

A. \( \text{K}_2\text{HPO}_4 \quad 6\, \text{g} \)
   \( \text{KH}_2\text{PO}_4 \quad 4\, \text{g} \)
   Dissolve in 100 ml distilled water and autoclave (121° C, 20 minutes)

B. \( \text{MgSO}_4 \cdot 7\text{H}_2\text{O} \quad 0.2\, \text{g} \)
   \( \text{NaCl} \quad 0.1\, \text{g} \)
   \( \text{CaCl}_2 \cdot 2\text{H}_2\text{O} \quad 0.26\, \text{g} \)
   \( \text{NH}_4\text{Cl} \quad 1.0\, \text{g} \)
   \( \text{L-malic acid} \quad 2.5\, \text{g} \)
   \( \text{NaOH} \quad 3.0\, \text{g} \)
   \( \text{Yeast extract} \quad 0.1\, \text{g} \)
   \( \text{FeCl}_3 \quad 10.0\, \text{mg} \)
   \( \text{NaMoO}_4 \cdot 2\text{H}_2\text{O} \quad 2.0\, \text{mg} \)
   \( \text{MnSO}_4 \quad 2.1\, \text{mg} \)
   \( \text{H}_3\text{BO}_3 \quad 2.8\, \text{mg} \)
   \( *\text{Cu(NO}_3)_2 \cdot 3\text{H}_2\text{O} \quad 0.04\, \text{mg} \)
   \( \text{ZnSO}_4 \cdot 7\text{H}_2\text{O} \quad 0.24\, \text{mg} \)
   Dissolve in 900 ml distilled water and autoclave (121° C, 20 minutes)
   Aseptically combine parts A and B when cool

* \( \text{CuSO}_4 \cdot 5\text{H}_2\text{O} (0.04\, \text{mg}) \) substituted for \( \text{Cu(NO}_3)_2 \cdot 3\text{H}_2\text{O} \)
APPENDIX C

Phosphate Buffer

Solution A: 27.8 g NaH$_2$PO$_4$ in 1000 ml distilled water
Solution B: 53.65 g Na$_2$HPO$_4$·7H$_2$O or 71.7 g Na$_2$HPO$_4$·12H$_2$O in 1000 ml distilled water

0.1 M of solution A and solution B were used as stock solutions. They were made as follows:

Stock A: 5.3 ml of solution A diluted to 200 ml
Stock B: 94.7 ml of solution B diluted to 200 ml

To make up 0.01 M phosphate buffer solution:

2.5 ml of each stock solution was diluted to 500 ml, adjusted to pH 7.0 and autoclaved at 121°C for 20 minutes. The final buffer solution was made up by mixing 78 ml of diluted stock solution A with 122 ml of diluted stock solution B.
APPENDIX D

Minus Nitrogen Nutrient Solution (Hoagland and Arnon 1938)

0.5 M $\text{K}_2\text{SO}_4$ 5.0 ml
1.0 M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 2.0 ml
0.05 M $\text{Ca(H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ 10.0 ml
0.01 M $\text{CaSO}_4$ 200.0 ml
Microelements 1.0 ml
Ferric solution
Made up to 1 liter with distilled water

Microelement Solution

$\text{H}_3\text{BO}_3$ 2.5 g
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 1.5 g
$\text{ZnCl}_2$ 0.1 g
$\text{CuCl}_2 \cdot \text{H}_2\text{O}$ 0.05 g
$\text{MoO}_3$ 0.05 g
Made up to 1 liter with distilled water

Ferric Tartrate Solution

$\text{FeCl}_3$ 5 g
Tartaric Acid 5 g
Made up to 1 liter with distilled water
LITERATURE CITED


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