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DEPARTMENT OF SCIENCE

MINISTRY OF ECONOMIC AFFAIRS

BANGKOK, THAILAND.

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TOA LABANUKROM Ph. D., Director General

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CONTENTS

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Study of Gold Ores from Toh Moh, Krabin and Ta Tago

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FOREWORD.

Gold deposits have been found throughout Thailand. Out of seventy provinces gold has been reported from twenty eight. Many deposits known are, however, geological freaks. In the past there were concessions to work for gold at Lomsakdi (หล่มศักดิ์), Ta Tago (ท่าตะโก), Bangsaparn Yai (บางสะพานใหญ่), Krabin (กระบินทร์), and Watana (วัฒนา). All the mine operations resulted in failures, but nothing is known of the cause. At present, there is only one gold mine in operation in this country; the Litcho Mine in the province of Naradhivas.

The geology of the gold fields just mentioned has not been worked out in details. With samples of ores and country rocks collected we studied the deposits morphologically and microscopically It is strongly hoped that this study, when completed, will be of some help to prospectors looking for gold deposits in Toh Moh, Krabin and Ta Tago.

GOLD ORE FROM TOH MOH.

Toh Moh is a district in the province of Naradhivas. It is a hilly district without adequate transport facilities. The deposit is at Ban Pajo and Litcho near the Malay boundary. It is much more convenient to get to the deposit by way of Kelantan.

Roughly speaking, the gold belt occupies an area extending from Tanyong Mas to the Malayan gold belt at Toh Moh. It is an area of schist intruded by fine grained granite. Dykes of biotitite are found intruding both rocks.

There are lodes in both granite and schist. The ore examined has been obtained from a lode worked by the Société des Mines d'or de Litcho striking N15°E and dipping 20°E. The lode is wholly in granite and traversed by many fault planes (rock slips) which make mining difficult. The ore body is about 3 feet wide and carries from eleven to fifty grams of gold per ton of rock.

Examined by naked eye the lode consists mainly of quartz which is both white and grey. There are fissures in the quartz; and in the fissures gold, pyrite, chalcopyrite, galena, sphalerite and arseno-pyrite are seen. The fissures are filled with greenish minerals which effervesce, in part, with dilute hydrochloric acid. Intermingled with the ore are pieces of greenish rock apparently placed inside the lode.

From eye examination, it is evident that gold was introduced later than the quartz of the lode and that some of it is embedded in sphalerite and pyrite. The gold is, in many places, very fine and scarcely perceptible to naked eyes.

A slide of the greenish rock found in the lode shows it to be a granite sheared and intruded by a hydrothermal solution causing alteration of feldspars to sericite. The quartz of the granite was corroded. Minerals brought with the solution include calcite and quartz.

Considering that the wall rock is granite, this greenish material inside the lode should be a horse rock, that is: a fragment of granite enclosed by quartz of the lode.

Slides of the vein quartz show that it had been intruded by epidote-calcite-quartz strings or veinlets carrying gold and the metallic sulphides. Original quartz crystals have been cracked and the boundaries corroded by the solution with the development of aggregates of tiny quartz crystals filling the spaces. In places, gold has been intruded into such cavities formed. Therefore, if the cracks extend far into the original mass of quartz, gold and the other sulphides may be present far from the veinlets. With naked eyes, this happening is not apparent; and the mistake might be made that gold also occurs in the mass of quartz, being seen apparently within it.

In the veinlets, there is seen something like a flow structure. Most of the gold occurs at the rim of the veinlets, very little appearing in the middle. Some of it occupies cracks in or around quartz crystals. The sulphide minerals are similarly placed. The kinds of minerals in the veinlets prove the deposit to be of mesothermal origin (i. e. one formed at medium temperature and under an intermediate thickness of rocks), although the original quartz vein in granite had been formed under hypothermal condition (i. e. high temperature and pressure).

With regard to the mineraliser, granite, being well consolidated before the breaking of the quartz vein in it, cannot be responsible for the cause of such mesothermal solution. The rest of the igneous rock is biotitite and the mine's geologist considered it to be the mineraliser. A gold assay of this rock has been made and it gives a value of less than 4 grams of gold per ton of rock, and so it can't be the mineraliser as thought of.

The study has shown definitely that the gold bearing solution came much later than the original quartz of the lode and is not in anyway genetically related to it. Before the incoming of such hydrothermal solution (hot watery juice) there should be a general earth movement, (a movement which affects all rocks within the area) which is responsible for the occurrences of faults and fractures in both the lode and the country rock. As gold is found in the fractures in quartz, it should also be found in fracturing zones of both granite and schist.

Gold Ore From Krabin (กระบินทร์)

The samples of ore and rocks were obtained from Ban Bo Tong (บ้านบ่อทอง) near Nong Sung station (สถานีหนองสัง). The area is within easy reach from the Eastern railway line; in fact it is the most accessible of all ore deposits in this country. Ban Bo Tong (บ้านบ่อทอง) is in the district of Krabin Buri (กระบินทร์บุรี). It is a flat country but well drained.

The deposit is in a gold belt extending from Lerya (เลย) to Aranyaprades (อรัญประเทศ). In this belt limestone is predominant and it is intruded by granites, diorites and porphyries, causing metamorphism in many parts of the region.

At Ban Bo Tong (บ้านบ่อทอง) gold has been found in a quartz vein in limestone striking East-West. From the vein a rock specimen had been obtained. Samples of the lode and wall rock were taken.

On inspection by naked eye the lode consists mainly of quartz, well crystallised ; in places, good crystals are seen. Probably the lode is vughy (full of cavities lined with quartz crystals) with crystals of quartz of the high temperature variety protruding into the vugh (cavities in ore veins). Gold is apparently embedded in the quartz. Nuggets of gold are common in this lode and have given an erroneous understanding that the lode is exceptionally rich. The contact rock consists of a green mineral, quartz and calcite. Gold has been spotted among the green mineral also.

A slide of the contact rock has been examined petrologically and was found to consist of calcite, quartz, green calcium garnet and wollastonite. The garnet is yellowish green and occurs mainly as rhomb-dodecahedral crystals. By qualitative analysis, the garnet appears to be a silicate of aluminium, iron and calcium. So it is a cross between glossularite and andradite. With crossed nicols the garnet gives anomalous colour interference with an isotropic core. It has been observed generally that in Thailand most of the calcium garnets formed by contact metamorphism are not isotropic. Wollastonite occurs in minute radiating needles in both quartz and garnet. By its acicular habit the mineral may as well be sillimanite.

The polished section of a specimen of ore near the wall has shown that gold is the latest mineral to crystallise so that it occupies spaces around the boundary of quartz crystals. Gold is also later than calcite. Some quartz is included in the gold, and tiny specks of gold have been seen inside quartz crystals. Therefore quartz and gold must have come together, the calcite belonging to the limestone. No calcite has been found in the middle of the vein.

The igneous rock found in the vein has been found to be a hornblende-diorite porphyry. Hornblende occurs as porphyritic crystals (large crystals on a fine mass) in a dioritic mass. Phenocrysts (large, well-formed crystals) of plagioclase feldspar are not clearly seen in the hand specimen, but under the microscope they appear as clear sections with zonings and striation twinnings. Specks of pyrite have been found in the rock as accessory constituents.

With the presence of hornblende-diorite porphyry, side by side with the gold-bearing quartz, it may be assumed that the rock is the actual mineraliser (one that comes along side with ore-bearing solutions). Owing to the presence of green garnets at the wall, the vein is evidently of high temperature but association with dyke rock depicts medium pressure. So the deposit is one of high temperature and medium pressure.

Gold Ore From Ta Tago (ท่าตะโก)

Southeast of Paknam Poh (ปากน้ำโพ) in Nakorn Swan (นครสวรรค์) is a village called Ban Bo Tong (บ้านบ่อทอง). The locality is in Umpur To Tago (อำเภอท่าตะโก) and may be approached from both Chan Sane (จันเสน) and Ban Mi (บ้านหมี่) stations. There is a cart track from Lopburi passing through Umpur Kok Sum Long (อำเภอโคกสำโรง) to the spot. Transportation is rather difficult, but in dry season a car can be taken there from the town of Lopburi (ลพบุรี).

This place has been known to be gold bearing for more than fifty years ago. It is surrounded by limestone hills of medium altitude. The country is gently undulating and practically dry. There are numerous streams, but in the absence of rain they are all dry. Natural springs occur in many places.

The geology is simple. It consists of an intrusion of quartz-monzonite porphyry into limestone. Both rocks were intruded later by quartz-diorite. Gold lodes occupy fault fissures in the porphyry.

The quartz-monzonite porphyry consists of an equal amount of plagioclase and orthoclase pheno-crysts in a ground-mass of monzonitic composition. Other phenocrysts include quartz and hornblende which in places predominate over all others. Magnetite occurs as an accessory mineral.

The intruding rock occurs in the gold vein and, examined by eyes alone, it appears to be spotted with black patches. Pyrite is an accessory constituent of the rock. It is found in the lighter mass but close to dark minerals. Petrological study has shown the rock to be a quartz-diorite consisting of andesine felspar, quartz, hornblende and some pyroxene. Some feldspar crystals are set in larger crystals of quartz. The structure suggests that quartz is the last mineral to crystallise. Magnetite is present as an accessory mineral. The black patches appear to be xenoliths of shale physically altered to magnetite and chemically changed to crystallo-blasts of orthoclase feldspar, hornblende, chlorite and biotite. The shale has been found under the limestone and, at the contact between the porphyry and limestone, shale masses have been found enclosed in the mass of porphyry. So the dark patches are without doubt altered shale masses digested by the quartz-diorite.

The lodes are of two types, white and green. White lode is a mixture of quartz and calcite, the latter being well crystallised. Pyrite is present in a considerable percentage and some gold has been found in it. The green lode is composed mainly of green, yellowish green and dark green calcium garnets. Calcite is present, admixed with the garnets in small amount. Examined by eye, the garnets crystallise in two habits: the rhomb—dodecahedral and the trapezohedral. It has been observed that crystals smaller than half a millimetre have rhomb-dodecahedral habit while larger crystals acquire the trapezohedral one. Larger crystals occur where there is calcite. Pyrite also occurs in contact with calcite. After dissolving the calcite out the garnet crystals may be seen to be formed inside the hollow as it is a vugh (cavity). Therefore it may be reasoned that garnet is the first mineral to crystallise while calcite and pyrite crystallise later to occupy vughs left by the crystallisation of garnet. In this portion garnet can crystallise under least resistance and therefore the crystal is large and of trapezohedral type. In the mass only rhomb-dodecahedral crystals are present because in such form the crystals grow easier. A little pyrite and calcite are embedded in the crevices between garnet crystals.

Gold is seen, in polished section, to crystallise later than the garnet so that it occupies the boundary between garnet crystals; therefore it is in the form of thin concave plates. Rarely, it has been found to crystallise in the cubic system with paralled cubic growth. Garnet crystals have been seen in gold and tiny specks of gold have been seen inside garnet crystals. It is a rarity that gold is present where the garnet crystals are large. The same thing has happened with vughy gold lodes of the quartz type where gold has never been found in the vugh. The presence of gold in garnet and garnet in gold proved that before the lode existed both were in the same melt.

The petrological study of the green garnet ore is extremely interesting. Calcite appears also in the centre of granet crystals, showing a reaction between silica and calcium carbonate to form the garnet. Where the section is greenish brown the garnet is completely isotropic, but, if colourless or greenish, the garnet seems to be composed of anisotropic zones arranged parallel to the crystal faces. It is a common occurence that isotropic and anisotropic zones are arranged in successions. The anisotropic zones are divided into layers meeting at the bisectors of the facial angles of the garnet crystal, clearly seen even under ordinary light.

Garnet crystallising in the trapezohedral habit is nearly completely isotropic. The section is octagonal with small anisotropic zones also with octagonal boundary near the rim of the crystal. The anisotropic zones are composed of layers parallel to the octagonal sides. Each set of layers meets at the bisectors of the facial angles of the crystal. Four sets, being parallel and perpendicular, extinguish in the same position (i. e. parallel to either layer). The colour of the various zones is, according to the rule found out above, greenish brown in isotropic zones and greenish in anisotropic zones.

Thick sections of the rhombohedral crystals show them to be divided into three parts representing each face of the rhombs. These faces are anisotropic and, when the stage of the microscope is rotated with the nicols crossed, each face extinguishes in successions. In thin sections, the boundary of each crystal is seen to be hexagonal with an isotropic hexagonal core. The bisector of the angles divide the crystals into six portions, alternate ones of which extinguish in the same position. Each zone is again divided into layers parallel to the hexagonal boundary.

Rarely a square cross section has been seen with a square isotropic centre. The crystal extinguishes in the position parallel to either side. This is so because the four zones formed by the bisectors of the angles are parallel or perpendicular. Similarly the zones are composed of layers parallel to the sides. It is an extreme rarity that garnet should crystallise with a cubic habit.

Qualitative chemical analysis has proved the garnet to be a silicate of aluminium, iron and calcium. So it is a cross between glossularite and andradite with a composition approaching that of andradite. The density of large isotropic trapezohedral crystals is about four and this is very near to the density of andradite.

The study has shown that the garnet has been formed by the interaction between calcium carbonate and silica under high temperature. Each crystal is composed of pyramids whose bases coincide with the faces. Each pyramid behaves as one optical unit.

The anisotropic pyramid shows successive growths or layers similarly orientated inside it. These layers give low interference colours in yellows and greys with straight extinction. They are biaxial, sometimes uniaxial, and optically negative. Therefore we think the garnet is composed of pyramids with compositions and physical properties of idocrase. This may be possible, considering that idocrase occurs also in contact metamorphic deposits like garnet. There might be conditions controlling the occurrences of the two minerals, and, when the two set of conditions overlap one another, both minerals are formed as one. The latter explanation failing, the mineral may be just taken to be a solid solution of andradite and glossularite.

In contact with the ore is a rock which has been determined petrologically to be composed of plagioclase feldspars, epidote, hornblende and pyroxene.

Some specimens of the white ore have shown that green garnet has been formed from it. Therefore the occurrences may be interpreted thus: movement of the limestone had caused the intrusion of quartz-monzonite porphyry which at the contact had dragged pieces of shale or slate under the limestone with it. Later on there was another movement resulting in a Northwest-Southeast fold. The porphyry was faulted in many places along the same direction. The fault zones were occupied by pyrite, quartz and a lot of calcite of mesothermal origin. Soon afterwards, quartz-diorite carrying gold and pyrite broke into the same fissure causing the occurrence of gold-bearing garnet ore. The heat of the quartz-diorite caused the reaction between originally existing quartz and calcite. Silica, leached from the intruding rock, attacked the remaining calcite to form garnets. Gold and pyrite accompanying the silica then occupied crevices between garnet crystals. The residual rock, after the silica had left, remained with the green garnet ore.

Evidently the deposit was formed under medium pressure and high temperature. The green garnet ore has a mean density of 3.3 and therefore is without doubt the world's new and densest gold ore. It has been proved for the first time also that gold has been carried in a solution alongside with quartz-diorite.

Conclusions.

(1) At Licho (ลี่ชอ) and Pajo (ปาโจ) villages in the Toh Moh (โต๊ะโม๊ะ) district gold occurs in quartz vein in granite and schist. A gold lode in granite has been found to be associated with biolitite. The gold has evidently been introduced with a hydrothermal solution containing quartz, epidote, calcite and sulphides of commonmeta ls into fissures in original quartz of the lode. Granite is sericitised (changed with the development of white mica) by the solution. The

deposit is of mesothermal (medium temperature and pressure) character.

(2) A gold lode has been found at Ban Bo Tong (บ้านบ่อทอง) in Krabin Buri (กระบินทร์บุรี). The deposit is in limestone and was formed under medium pressure and high temperature. Quartz is the main gangue and it is well crystallised. Gold has been found to have come with the quartz, which was leached from a hornblende-diorite. porphyry being found in the same vein. High temperature of the intrusion has caused metamorphism of the limestone wall with development of green garnet and wollastonite.

(3) A gold deposit at Umpur Ta Tago (อำเภอท่าตะโก) in Nakorn Swan (นครสวรรค์) has been formed by the instrusion of a quartz-diorite into an original mesothermal quartz-calcite vein with gold in quartz-monzonite porphyry. The ore consists mostly of green calcium garnet. Calcite, pyrite and gold are present in the garnet. The ore is new to the world and it has been proved that it is the quartz-diorite that came alongside with gold solution. The deposit was formed under medium pressure and high temperature.

It may be mentioned here that in this part of the world gold has been found to be associated closely with rocks of the diorite family. In Burma and also in the Philippine Islands gold is associated with diorites and andesites. Some gold in Thailand has been found near granite intrusions but the occurrence is infrequent.

Detailed geological studies of the three deposits are forth coming, and all ideas arising from the microscopical studies have yet to be proved by definite geological reasonings.

Fig. 1. Krabin Gold Ore, showing white patches of gold enclosing quartz. Black spots are calcite, grey quartz and white specks of gold.

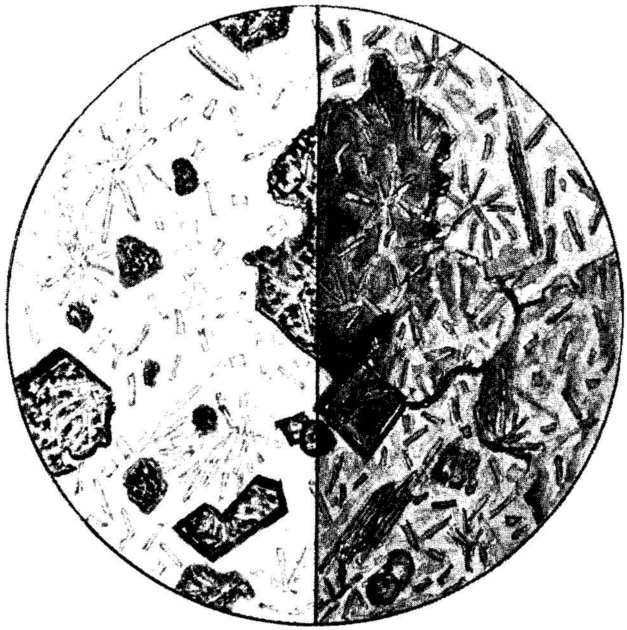

Fig. 2. Contact rock from Krabin, showing development of green garnet and wollastonite in a mass of quartz and calcite.

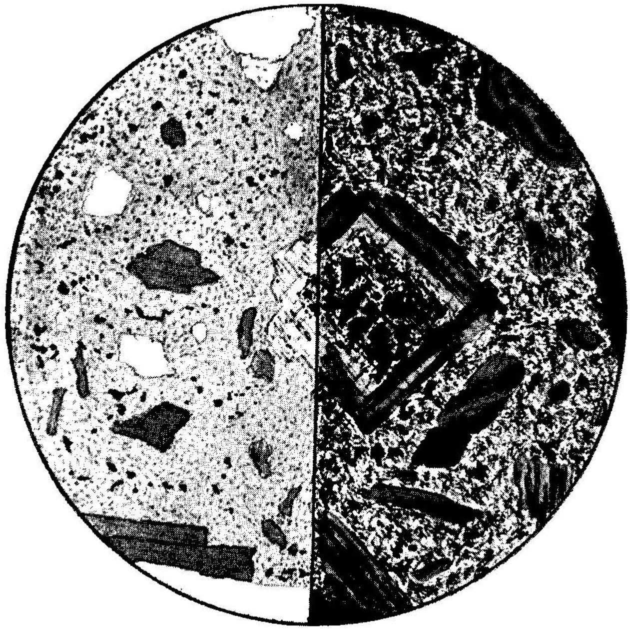

Fig. 3. Hornblende-diorite porphyry from Krabin, showing phenocrysts of feldspar and hornblende on a dioritic groundmass.

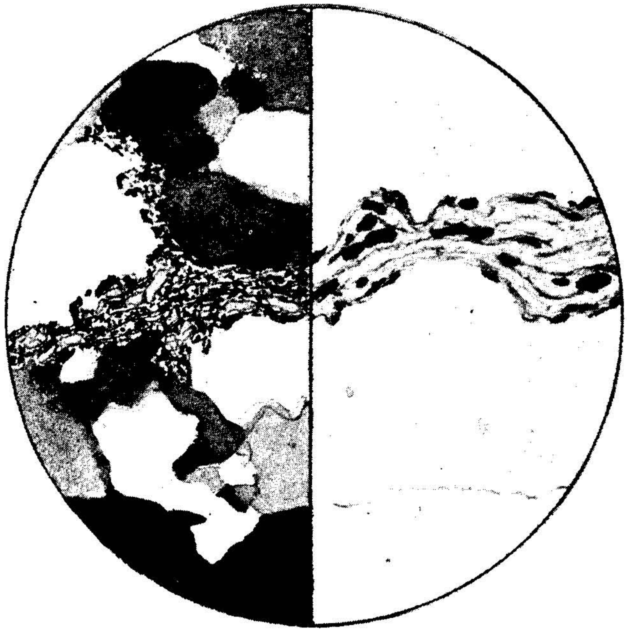

Fig. 4. Quartz ore from Toh Moh showing intrusion of gold bearing solution containing epidote, calcite, pyrite and quartz into the original quartz of the vein. The gold is shown as black spots.

Fig. 5. Sericitised granite horse rock in gold lodes from Toh Moh. Note the alteration of feldspar and corrosion of quartz crystals.

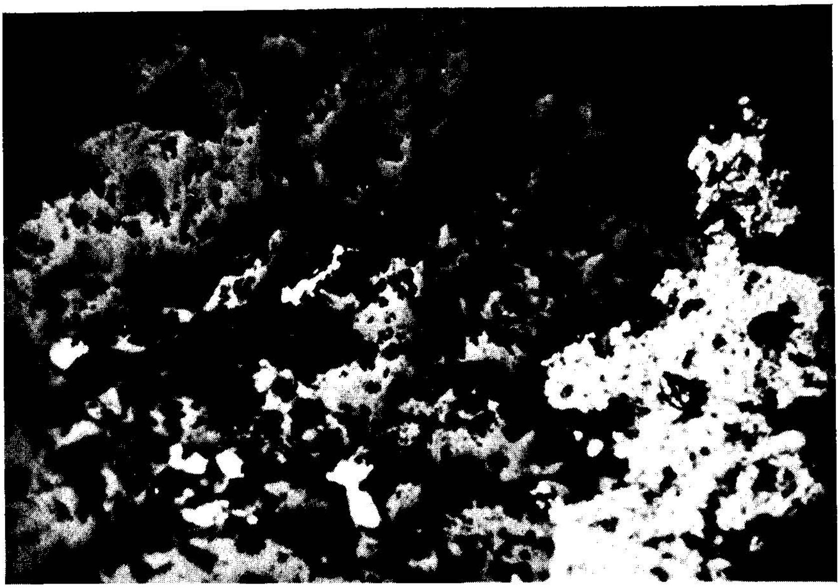

Fig. 6. Ta Tago green garnet ore showing gold in white patches, occupying interstices between garnet crystals, shown in grey and dark tones.

Fig. 7. Zoning in Ta Tago garnet. The central portion is green and anisotropic, the next zone brown and isotropic, and the last green and anisotropic.

Fig. 8. Quartz-Monzonite Porphyry from Ta Tago, showing phenocrysts of plagioclase feldspar, quartz and hornblende on a ground mass of monzonitic character.

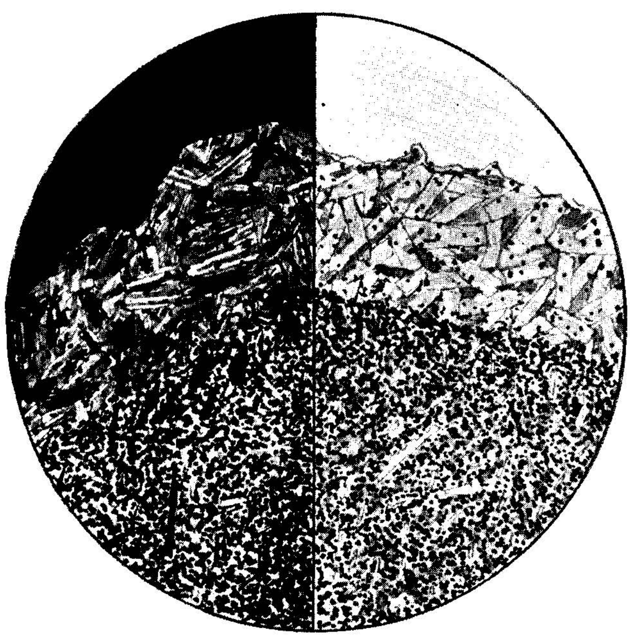

Fig. 9. Quartz diorite with altered shale from Ta Tago. Black spots are magnetite.

Fig. 10. Gold bearing garnet ore from Ta Tago, showing octagonal, hexagonal and square cross sections of garnets between crossed nicols.

The Relation of Varieties of the Soybean to Various Strains of the Rhizobia.

BY

Thongjaya Punyasingha

Of the Division of Agricultural Science, Department of Science, Ministry of Economic Affairs, Bangkok, Thailand.

INTRODUCTION.

Variation in nodule formation among different varieties of soybeans (Glycine max (L) Merr) has been reported from different parts of the United States. In many cases a certain variety has failed to show inoculation when treated with a culture of known origin, while among different varieties growing side by side one has been inoculated and the other has remained free or practically free of nodules.

Leonard (16) concluded that a culture of bacteria isolated from a single variety of soybeans will form nodules on other varieties. In his experiments the organisms were isolated from the medium yellow variety of soybean.

The ability to form nodules varies among the different kinds of soybeans. Apparently certain varieties are more easily inoculated than others. It is also true that the time of ripening of one variety may influence the infection and subsequent formation of nodules. For instance, an early and late variety may show differences in inoculation as a result of variation in plant food at different times of the year. Repeated investigations have proved that the formation of nodules is influenced to a considerable degree by the reaction and salt concentration of the soil water as well as by other factors.

This paper is the thesis submitted by Thongjaya Punyasingha, now in the Division of Agricultural Science, Department of Science, Bangkok, Thailand, in partial fulfilment of the requirements for the degree of Master of Science of Cornell University, U.S.A., in September 1939.

Kirchner (15) called attention to the fact that, of about 100 species of legumes growing in the botanical garden at Hohenheim, only the soybean failed to develop nodules. He believed that this was due to environmental conditions. Later he obtained some soil from Japan which contained the organism for soybean, and in some pots and field experiments with two varieties of soybeans proved definitely that soybean bacteria were different from other legume bacteria and that the soybean would produce nodules by using soil from a field in which soybeans bearing nodules had been grown. Kirchner was probably the first investigator to use soil for the inoculation of soybeans.

In 1903 Hiltner and Strömer (13) made the first report on soybean inoculation experiments, using pure cultures. In one of their experiments similar quantities of Dahlem soil and unlimed and limed moor soils were inoculated with a pure culture of soybean bacteria. The best inoculation was secured with the limed moor soil, while with the unlimed moor soil the inoculation was very poor. In every case the best inoculation was secured when the largest quantity of soil was added.

Atwater and Woods (4) suggested the use of soil infusion for the inoculation of soybeans. They carried out sand culture experiments, but the results were not very satisfactory. Of the inoculated plants, about one-third did not show any nodules, while the remainder had a very few small tubercles.

Moore (18) stated that out of a total of 129 reports received from the soybean cultures sent out by the U. S. Department of Agriculture, 43 per cent of the tests were failure; more failures being reported for soybeans than for any other crop.

Lipman (17), in experiments with Farmogerm and Nitragin, showed that, on soils well drained and properly supplied with moisture, lime, phosphates, and potash, these cultures were capable of increasing the yields of such leguminous crops as had not been previously grown on the land.

Fellers (8) found that many of the commercial cultures tested failed to give satisfactory results with soybeans. He recommended the soil transfer method for this crop, except when the commercial cultures were known to be of good quality.

Later, Feller (9) compared the relative efficiency of commercial cultures and nodule infusion for the inoculation of soybeans and reported that the cultures gave just as good results as the nodule infusion. Kendal (14), using four commercial cultures for soybean inoculation, found that the inoculated plants showed a remarkable difference in color and also in the number of root nodules when compared with uninoculated plants.

In 1924, Fiske (11) reported the official test on 33 samples of legume inoculants. Five were either fair or poor, while all the rest produced good inoculation.

Hiltner and Störmer (13) described the two methods, proposed by Nobbe and Hiltner in 1896, for using nitragin. In one method, called soil inoculation, the culture was mixed with a quantity of soil which was then spread over and worked into the the surface soil. In the other method, called seed inoculation, the seeds were moistened with the melted gelatin culture and then mixed with a small quantity of sand or soil to absorb the excess moisture and to prevent the seeds sticking together. In the majority of the field tests reported the soil inoculation method gave the better results. In an experiment in which inoculation was accomplished by mixing a pure culture and water with forest humus soil, with compost soil with Dahlem soil and with quartz sand, plants taken from the quartz-sand-pure-culture inoculated plots average 11 nodules per plant, while all the others averaged less than 2 nodules per plant. It was concluded that these soil media did not serve as suitable carriers for the soybean bacteria.

In another field experiment, comparing the efficiency of a pure culture with that of a culture prepared by crushing fresh nodules in water, the same investigators found that soaking the seeds under water for five hours before applying the pure cultures increased the nodulation almost four times. Soaking the seeds under water before inoculation also led to better results when the nodule infusion was used. The pure culture gave a much better nodule formation than the infusion prepared from crushed nodules.

Otis (21), in inoculation tests with two varieties of soybeans in Kansas, used soil from Massachusetts, applying one-twentieth of a pint in each hill or pouring in one-sixth of a pint of a soil extract prepared by stirring thoroly 1 part of soil to 7 parts of water and allowing the soil to settle. Where the soil extract was used the tubercles on the Yellow Soy were numerous and well developed, while those on the medium Green were scantly and rather inferior. Where the soil was used the two varieties were similarly nodulated. In a later pot culture experiment with four other varieties of soybeans no differences were noted between the soil inoculation method and the soil extract of inoculation method. Increasing the amount of soil from one-twentieth of a pint to one and one half pints per pot of soil did not produce any better nodulation. Successful inoculation was secured by using nodulated roots that had been left in the soil for about a month. When the nodulated roots were washed and air-dried for about a month before being used, much less satisfactory nodulation occurred.

Garman and Didlake (12) found that soybean seeds soaked overnight in a liquid suspension of the soybean bacteria were 100 per cent inoculated, while shorter periods of soaking were less effective. In another experiment in which the seeds were soaked and dried overnight and planted the following day, over three-fourths of the plants had nodules. When the inoculated seeds were not planted for four days, one week and two weeks, much less inoculation occurred and, when three weeks elapsed before the seeds were planted, no nodules were present.

Fellers (10) inoculated soybean seeds with a nodule infusion and found that viable organisms remained on the seed coats for six to nine months. He showed that the bacterial cells were most rapidly destroyed the first few hours after inoculation.

Noyes and Cromer (20) found that one pound or one-half pound of soil per acre was insufficient to inoculate soybeans. Four commercial cultures used in the proportion required per plant according to directions did not give satisfactory nodulation. Applying a commercial culture at double the rate gave an average of 75 per cent of nodulated plants against 20 per cent for no commercial inoculation.

Arny and McGinnis (3) found that, where water alone or 5, 20, or 30 per cent glue and sugar solutions were used with soil as the inoculant, the plants generally were very slightly inoculated. Equal amounts of soil and seed gave satisfactory inoculation, while the commercial culture with few exceptions, gave similar results. Storing the seed for a short time, or exposing the soil to the sun for one-half hour, did not affect the inoculation.

Nightingale (19) studied the soil, water and milk methods of applying the bacteria to the seed and found that the soil method gave the best results, although there was little difference between the soil and water methods.

Perkins found that the maximum nodulation of Virginia soybeans was secured when the number of infecting organisms was between 25 and 30 per seed. A rather definite number of organisms was required to produce maximum infection, and after a certain degree of infection was reached, the plant was immune to additional infection.

Alicante (1) inoculated soybean seed with a bacterial infusion alone and with the bacterial infusion plus soil, or glue, or sugar and combinations of these materials. Some of the seeds were then planted immediately; others were planted at regular intervals, and the last planting was made 2 months after inoculation. Nodule production was found to be fairly consistent, regardlesss of conditions and kind of treatment. The time of storage up to two months after treatment did not seem to influence nodule production. The use of sugar with the infusion was superior to the addition of glue or soil, the soil proving of some benefit but the glue having very little effect.

Voorhees (24) inoculated several varieties of soybean with cultures of Nitrogerm and Farmogerm and found considerable difference in the nodulation obtained on the different varieties. He concluded that different and definite powers of resistance to association with the symbiotic bacteria were measurable.

Leonard (16) inoculated 19 varieties of soybeans with a pure culture of bacteria isolated from the Medium Yellow soybean and secured inoculation in all cases.

Fred and Bryan found that pure cultures of organisms from Mammoth Yellow, Medium Green, Manch and Haberlandt soybeans could be used interchangeably on these varieties, and the same was true for soil inoculation. Other field tests with additional varieties gave similar results. No evidence was obtained to justify the conclusion that the nodule bacteria are specific.

The accumulated data indicate that the ability to form nodules varies among the different kinds of soybeans. Apparently certain varieties are more easily inoculated than others. It is also true that the time of ripening of one variety may influence the infection and subsequent formation of nodules. For instance, an early and late variety may show differences in inoculation as a result of variation in plant food at different times of the year. Repeated investigations have proved that the formation of nodules is influenced to a considerable degree by the reaction and salt concentraction of the soil water as well as by other factors.

EXPERIMENTAL.

Isolation of Cultures.

The seeds of the soybean employed were sterilized in a solution of calcium hypochlorite (Wilson 26). The length of time which the seeds were treated varied with the different species of seed and with the treatment which was given to the seed to permit them to absorb water. In general, a solution of about ten per cent of calcium hypochlorite was allowed to stand on the seed from fifteen to thirty minutes. The seeds were then washed with sterile water and placed in Erlenmyer flasks which contained sterile sand where they were allowed to dry before the inoculation was added. The inoculation was added in the solution which was used to wet the sand. The solution contained 0.05% KH_2PO_4 and 0.05% sucrose. Twenty cc. of solution were added for each hundred grams of sand.

Within three to five weeks after planting, a well defined contrast was noticeable in size and color of the inoculated plants. The uninoculated plants were smaller and at the end of four to five weeks decidedly yellow. At this stage the plants were removed from the flasks and examined for nodules. For reisolation of the organism the nodules were immersed in a solution of calcium hypochlorite, in which the odor of chlorine was barely detectable, for three to five minutes, depending on the size of the nodule. The nodules were then washed in sterile water, and placed in a petri dish containing a few drops of sterile water. The nodules were crushed with a sterile spatula. Loop dilutions were made from the macerated nodule to three other petri dishes which also contained a few drops of sterile distilled water. Approximately ten cc. of Ashby's (2) medium was then added to each petri dish.

The composition of Ashby's medium is :—

Manitol ($C_6H_8(OH)_6$)	... 10.0	gr.
Monopotassium phosphate (KH_2PO_4)	... 0.2	,,
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	... 0.2	,,
Sodium chloride ($NaCl$)	... 0.2	,,
Calcium sulphate ($CaSO_4 \cdot 2H_2O$) ...	... 0.1	,,
Calcium carbonate ($CaCO_3$) ...	... 5.0	,,
Distilled water	... 1,000	cc.

After incubating for one week at 25°C., transfers were made to slopes of the same medium. As soon as sufficient growth occurred, the culture were stained with saturated alcoholic gentian violet. The rhizobium remains unstained in most instances when water is not present. The back-ground is stained a deep gentian violet. All cultures which did not show this typical staining characteristic were discarded. Those which appeared to be pure cultures of Rhizobium when examined microscopically were checked for nodulation on the original host plant, as well as the plant from which they were last isolated. Those failing to symbiose with the plant were discarded.

In each series of plantings adequated controls were grown. In no case were nodules found on those plants which were not inoculated.

PRESENTATION OF DATA.

Symbiosis Between Various Strains of the Rhizobia and Soybean Plants.

In making this test of symbiosis between various strains of the rhizobia and soybeans, the varieties were obtained from the Department of Geneties, Cornell University. They were described very largely by serial numbers. These numbers together with the names of certain varieties are listed in Table 1.

The (+) signs in the squares in the table indicate that the plants and the bacteria were symbiosing as evidenced by the appearance of nodules. The (−) signs indicate no nodules were found on the roots of the plants.

TABLE 1.

Symbiosis Between Various Strains of the Rhizobia and Soybean Plants.

Varieties of Soybean	Strains of the Rhizobia								
	Albizzia julibrissin	Amphicarpa monoica	Apios tuberosa	Baptisia australis	Dalea alopecuroides	Glycine max	Lespedeza striata	Phaseolus vulgaris	Vigna sinensis
53933	—	+	—	—	—	+	—	—	—
54608	—	—	—	—	—	+ +	—	—	+
65341	—	—	—	—	—	+ +	—	—	+
79602	+	+	—	—	—	+ +	—	—	+
79620	—	—	—	—	—	+	—	—	+
79746	+	—	—	—	—	+ +	—	—	+
79773	—	+	—	—	—	+ +	—	—	—
85340	—	—	—	—	—	+	—	—	+
86023	—	—	—	—	—	+ +	—	—	+
88803	—	—	—	—	—	—	—	—	—
89154	—	—	—	—	—	+ +	—	—	+
90566	+	—	—	—	—	+	—	—	+
90579	—	—	—	—	—	+	—	—	—
92468	+	—	—	—	—	+ +	—	—	+
92634	—	+	—	—	—	+ +	—	—	+
92637	+	—	—	—	—	+ +	—	—	+

TABLE 1.

SYMBIOSIS BETWEEN VARIOUS STRAINS OF THE RHIZOBIA AND SOYBEAN PLANTS.

Varieties of Soybean	Strains of the Rhizobia								
	Albizzia julibrissin	Amphicarpa monoica	Apios tuberosa	Baptisia australis	Dalea alopecuroides	Glycine max	Lespedeza striata	Phaseolus vulgaris	Vigna sinensis
65344	+	—	—	—	—	+ +	—	—	+
92640	—	—	—	—	—	+	—	—	+
92681	+	—	—	—	—	+	—	—	—
92686	—	—	—	—	—	+ +	—	—	+
92716	—	—	—	—	—	+	—	—	+
92719	—	—	—	—	—	+ +	—	—	+
92791	+	—	—	—	—	+ +	—	—	+
92868	—	—	—	—	—	+	—	—	—
96194	—	—	—	—	—	+ +	—	—	+
96200	—	—	—	—	—	+	—	—	+
Bansei	+	+	—	—	—	+ +	—	—	+
Cayuga	+	+	—	—	—	+ +	—	—	+
Delicious	—	—	—	—	—	—	—	—	+
Dunfield	+	—	—	—	—	+ +	—	—	+
Early Michigan	+	+	—	—	—	+ +	—	—	+
Black Eyebrow	—	—	—	—	—	+	—	—	—

TABLE 1.

Symbiosis Between Various Strains of the Rhizobia and Soybean Plants.

Varieties of Soybean *	Strains of the Rhizobia								
	Albizzia julibrissin	Amphicarpa monoica	Apios tuberosa	Baptisia australis	Dalea alopecuroides	Glycine max	Lespedeza striata	Phaseolus vulgaris	Vigna sinensis
Hakkaido	+	+	—	—	—	+ +	—	—	—
Hiro	—	—	—	—	—	+	—	—	+
Kura	—	—	—	—	—	+ +	—	—	+
Manchu	+	+	—	—	—	+ +	—	—	+
Mandell	—	—	—	—	—	+ +	—	—	+
Minsoy	—	—	—	—	—	+	—	—	+
Osoya	—	—	—	—	—	+ +	—	—	+
Soja graciles	—	—	—	—	—	—	—	—	—
Shiro	—	—	—	—	—	+ +	—	—	+
Suru	+	—	—	—	—	+	—	—	+
Waseda	—	+	—	—	—	+ +	—	—	+
Wilson	—	—	—	—	—	+ +	—	—	+

* + + = 50% or more of plant nodulated.
+ = Less than 50% of plant nodulated.
— = No nodules.

Discussion.

Most of the early investigators were of the opinion that any strain of Rhizobium would symbiose with all leguminous plants, but that a given strain of bacteria may be better adapted to a species of leguminous plants than some other strain. Breall (5) reported nodulation of pea, lupine, and alfalfa with the bacteria isolated from alfalfa in 1888. Ward in 1889 reported cross-inoculation between peas and broad bean. In 1890-91 Laurent reported that he had been able to produce nodules on peas with thirty-six strains of Rhizobium isolated from as many plant species.

Prazomowski (22) was the first to show that a definite species of legumes will not symbiose with all strains of the rhizobia. He failed to obtain symbioses with lupine and Rhizobium isolated from pea nodules. Nobbe, Schmid, Hiltner, and Hotter confirmed and extended the work of Prazmowski to include organisms isolated from Pisum, Robinia, Cytisus, and soil in which Gledissia had grown. None of these organisms symbiosed with either the pea or bean bacteria, but not with lupine or Robinia bacteria.

Later investigators came to the conclusion that the specificity between bacteria and plant was definite enough to consider the strains of bacteria which symbiose with a particular plant or group of plants as a species of the root nodule bacteria. Fred, Baldwin, and McCoy published a review of the literature in which the various groups and species are discussed. They consider the early work which does not agree with this grouping as being of little value, due to faulty technique. Wilson (25), Raju (23) and others, using improved technique, have shown that the bacteria which are adapted to one host plant may symbiose with other plants, provided conditions are suitable. Wilson's work indicated that nearly all plants will symbiose with several strains of bacteria, although the percentage of plants infected may be very low. If a plant is grown in a soil which is devoid of bacteria adapted to that plant, it may symbiose with bacteria which are not well adapted to the plant.

Allen and Baldwin (2) have shown that bacteria from red clover which are not affective become more effective with each passage through the plant. Therefore, we may expect a flora which is adapted to a plant to develop, although the strains present when the seeds are planted may not be well adapted to that plant.

Burrill and Hansen (6) suggested that the ability of a strain of Rhizobium to symbiose with a certain host plant may be due to the enzymes or other differences in the root sap which cannot be determined by chemical methods. If this is true, it might be expected that growing the bacteria in the extract of a plant would cause them to become adapted to that plant in much the same way as when the bacteria are grown in the nodules of that plant. Burke and Hohl (7) obtained negative results with the extract of alfalfa and the bacteria from vetch and red clover. Their extract was obtained, however, by adding distilled water to the tissue and grinding in a mortar. Dilution might cause the effect of enzymes of the plant to be less intense than that in the nodules.

In considering the adaptation of a strain of Rhizobium to a legume, the variations in individual plants must be overlooked. The characters of the individual plant which permits symbiosis may be slightly different from the other individuals of the same species. This difference may be great enough to permit a poorly adapted strain to symbiose with one plant which all of the others growing under the same conditions may produce no nodules. During this symbiotude the characters of the bacteria are changed to such an extent that the bacteria will symbiose with a larger per cent of the plants on the next exposure.

On consulting the table in connection with symbiosis between various strains of the Rhizobia and soybean plants, it may be seen that the strain of Rhizobium from Glycine max seem more adapted to Glycine, Vigna, Albizzia and Amphicarpa in the order named.

CONCLUSIONS.

The conclusions drawn from the data presented in this paper may be summarized as follows:—

1. The plants with which a strain of Rhizobium symbioses vary according to the plants with which it has previously symbiosed.

2. Some cultures seemed to be more efficient in inoculating one variety than the others, but no single culture produced the best inoculation on all forty-four varities tested.

3. While some cultures were consistently good in all of the tests, others proved to be very inefficient for the inoculation of soybeans.

4. Indications that certain definite strains of soybean bacteria are required to produce the most efficient degree of nodulation on a given variety of soybean were obtained.

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BIBLIOGRAPHY.

1. Alicante, M. M.
The Viability of the Nodule Bacteria of Legumes Outside of the Plant, I, II. Soil Sci. 21 : 27-52. 1926.

2. Allen, O. N., and Baldwin, I. L.
The Effectiveness of Rhizobia as influenced by Passage through the Host Plant. Wis. Agr. Expt. Sta. Res. Bul., 106 : 1-56. 1931.

3. Arney, A. C. and McGinnis, F. W.
Methods of Applying Inoculated Soil to the Seed of Leguminous Crops. Jour. Amer. Soc. Agron. 13 : 289-303. 1921.

4. Atwater, W. O. and Woods, C. D.
The Acquisition of Atmospheric Nitrogen by Plants. Storrs Agr. Expt. Sta. Third Ann. Apt. 12-14. 1890.

5. Breal, E.
Observations sur les tubercles a bacteries que se developpent sur les racines des Leguinineuses. Ann. Agron. 14 : 481-495. 1888.

6. Burril, T. J. and Hansen, R.
Is Symbiosis Possible between Legume Bacteria and Non-Legume Plants? Ill. Agr. Expt. Sta. Bul. 202 : 115-181. 1917.

7. Burke, V. and Hohl, N. J.
Cross-Inoculation with Rhizobium radicicolum. Soil Sci., 30 : 407-411. 1930.

8. Fellers, C. R.
Report on the Examination of Commercial Cultures of Legume Infecting Bacteria. Soil Sci. 6 : 53-67. 1918.

9. Fellers, C. R.
The Effect of Inoculation, Fertilizer Treatment and Certain Minerals on the Yield, Composition and Nodule Formation of Soybeans. Soil Sci. 6 : 8-129. 1918.

10. Fellers, C. R.
The Longevity of B. radicicola on Legume Seeds. Soil Sci. 7 : 217-232. 1919.

11. Fiske, J. G.
Results of Seed and Legume Inoculant Inspection, 1924, Bul. N. J. Agr. Expt. Sta. 412. 1925.

12. Garman, H. and Didlake, M.
Six Different Species of Nodule Bacteria. Bul. Ky. Agr. Expt. Sta. 184. 1914.

13. Hiltner, L. and Störmer, K.
Neue Untersuchungen under die Wurzel-Knollchen der Leguminosen und deren Erreger. Arb. aus der Biol. Abt. fur Land. und Forst. am. K. Gendhtsamt. 3 : 151-307. 1903.

14. Kendall, J. C.
Report of the Director of the New Hampshire Agricultural Experiment Station for the Year Ending June 30, 1921. Bul. N. H. Agr. Expt. Sta. 203. 1922.

15. Kirchner, O.
Die Wurzelknollchen der Soja-bohne. Cohn's Beitr. zur. Biol. der Pflanzen (Breslau) 7 : 213-223. 1895.

16. Leonard, L. T.
Variation in Nodule Formation. Jour. Amer. Soc. Agron. 8 : 116-118. 1916.

17. Lipman, J. G.
Tests of Commercial Cultures for Soil Inoculation. Bul. N. J. Agr. Expt. Stra. 227. 1910.

18. Moore, G. T.
Soil Inoculation for Legumes. Bul. U. S. D. A. Bur. Plant Industry 71. 1905.

19. Nightingale, W. I.
Factors that Control the Infection of Legumes by Bacteria. Bul. Wash. Agr. Expt. Sta. 167. 1922.

20. Noyes, H. A. and Cromer, C. O.
Tests of Commercial Cultures for Legume Inoculation. Soil Sci. 6 : 69-79. 1918.

21. Otis, D. H.
Master's Thesis. Kansas State Agr. College. Reprinted in part in Bul. 96 of the Kansas Agr. Expt. Sta. 1896.

22. Frazmowski, A.
Das Wessen und die biologishe Bedsutung der Wurzelknollchan der Erdse. Ber. ad. Sitz. d. Akad. Wissensch. Krakau. Bot. Centbl., 39 : 356-362. 1889.

23. Raju, N. S.
Studies on the Bacterial-plant groups of Cowpea, Cicer and Dhaincha. I Classification. Zentbl. of Bakt., 2 Abt., 94 : 240-262. 1936.

24. Voorhees, J. H.

Variations in Soybean Inoculation. Jour. Amer. Soc. Agron. 7 : 139-140. 1915.

25. Wilson, J. K.

Physiological Studies of Bacillus radicicola of Soybean (Soja max Piper) and of Factors Influencing Nodule Production. Bul. N. Y. Cornell Agr. Expt. Sta. 386. 1917.

26. Wilson, J. K.

Calcium hypochlorites as a seed sterilizer. Amer. Jour of Botany, 2 : 420-427. 1915.

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