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Effects of inoculum concentrations on the resistance of peanuts to *Puccinia arachidis* Speg.[1]

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Abstract : Four hundred and sixty-one accesions of peanuts were screened for resistance to peanut rust. Three accessions, P. I. 314817, P. I. 259747 and P. I. 315608, showed slow rusting under natural environments in Taiwan. When these accessions were screened in a rust nursery with an inoculum density higher than that in the natural field, the P. I. 314817 and P. I. 259747 became susceptible while P. I. 315608 remained resistant. Heavy infection occurred on all three accessions when they were inoculated with a spore suspension (200 mg of uredospores/plant) plus a wetting agent (Tween 80) in growth chamber. In another inoculation tests, each of the three accessions at 90 days old was inoculated with inoculum densities ranged from 2 to 200 mg of uredospores/plant in greenhouse. The results revealed that each accession had produced different amount of spores and degree of infection. Density of inoculum plays an important role in the infection and symptom expression. No different physiological races could be identified based on their reaction types on P. I. 314817, P. I. 259747, P. I. 315608 and TN-4 for the fourteen isolates of *P. arachidis* from Taiwan.

Peanut rust caused by *Puccinia arachidis* Speg. was first reported in Taiwan by Kao and Wu in 1970. It now occurs widely all over the island. When peanut plants are heavily infected, the leaves turn yellow and defoliated, seeds are smaller, and the yield, then, is reduced to about 50% (Huang and Chen, 1979). No commercial variety of peanuts in Taiwan is resistant to this disease. Therefore, searching for sources of resistance is extremely important in breeding program.

Three peanut genotypes, P. I. 259747 (Tarapoto). P. I. 314817 (DHT 200) and P. I. 315608 (Israel Line 136) were reported resistant to the rust in most peanut growing areas in the world (Bromfield, 1971 ; Hammons, 1977 ; Subrahmanyam, *et al*, 1980). However, the resistance of these genotypes is still controversial. McVey (1965) reported that peanut

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accessions exposed to natural rust infection in Puerto Rico, only P. I. 259747 had good resistance. However, rust symptoms were observed on seedlings of this accession when inoculated with a spore suspension and incubated in a saturated atmosphere for 16-24 hr in greenhouse. Bromfield and Cevario (1970) made inoculation in a dew chamber or plastic tent and suggested that in addition to P. I. 259747, two more accessions, P. I. 314817 and P. I. 315608, were physiologically resistant to rust isolates obtained from Texas and Puerto Rico. They also pointed out that the number of pustules developing on these accessions was consistantly less than that on the susceptible variety 'Starr' in a given inoculation test. The two rust isolates could not be separated into two physiological races on the basis of reaction types on the host accessions. Cook (1972) reported that P. I. 314817 and P. I. 259747 were resistant, but P. I. 315608 was highly susceptible to rust in Jamaica. He also indicated that the resistance of P. I. 259747 increased as the plant aged. This was confirmed by Subrahmanyam, *et al* in 1980. Romero (reviewed in Hammons, 1977) found that among the genotypes tested in Honduras, P. I. 259747 showed resistance, but P. I. 314817 and P. I. 315608 were susceptible.

The reasonable explainations for the above controversy are : (1) the physiological races of peanut rust fungus exist in the world ; (2) the resistant accessions are not genetically uniform ; or (3) the accessions may possess horizontal resistance to this fungus with the amount of resistance expressed being dependent on the environmental conditions. This study was conducted to determine whether these accessions are resistant to peanut rust in Taiwan, the possible use of them in breeding program and to evaluate whether one or more of the three explainations offered above are ture.

Materlals and Methods

Field screenings.-Peanut seeds of P. I. 314817, P. I. 259747 and P. I. 315608 were supplied by Dr. G. A. White in Maryland, U. S. A. and another 458 accessions were obtained from Taiwan Agricultural Research Institute (TARI) germplasm collection. All 461 accessions were screened by natural infection in the field at TARI in 1979. A simple spore trap equipped with four slides was set up 30-cm above the canopy in the center of the plots. The slides were coated with gel and changed daily. The number of uredospores per 3.24 cm^2 was counted under microscope. The disease readings were taken at 7 to 10-day intervals throughout the crop seasons. The degree of infection was rated by an "intensity grade scale" from R_0 to R_4, based on the percentage of leaf areas covered with pustules. R_0 signifies no pustules on leaves, R_1 less than 25%, R_2 from 26 to 50%, R_3 from 51 to 75%, and R_4 more than 75% of leaf areas covered with pustules. Disease index 1 is given to R_1, 2 to R_2, 3 to R_3, and 4 to R_4. All leaves on the main stem of each plant were sellected to record the disease intensity and calculated by the followi ng formula :

$$\text{Disease intensity} = \frac{NR_1\times 1+NR_2\times 2+NR_3\times 3+NR_4\times 4}{(NR_0+NR_1+NR_2+NR_3+NR_4)\times 4}\times 100\%$$

N = Number of leaves recorded for each scale

The final score of disease intensity for each accession was obtained from the average

of 10 plants.

Rust nursery screenings.-A rust nursery was set up at TARI in 1979 by seeding many rows of the highly susceptible variety TN-4 every 20 to 30 days in a 0.3 ha field from Feburary to October. Forty pots of peanut plants infected with rust in the greenhouse were randomly distributed in the nursery as source of incoulum. Therefore, after the pathogen spreaded, the nursery could provide inoculum from May to December. The test accessions were planted, 50 cm between rows and 15 cm between plants within rows, in the nursery on May 8, 1979.

Greenhouse tests.-Fourteen isolates of *P. arachidis* collected from different peanut growing areas in Taiwan were used. Plants grown in pots were inoculated at 30, 60, or 90 days after seeding by spraying them as evenly as possible with uredospore suspension (0.1g/100ml) containing 0.1% of wetting agent (Tween 80). The inoculated plants were incubated in a transparent plastic tent for high humidity for 24 hr and then moved to a greenhouse bench at 25-30 C. The disease readings were taken 18 days after inoculation. Plants of 90 days old also were inoculated, 20 ml per plnat, with spore suspensions at 0.01, 0.05, 0.1, 0.5, or 1.0g/100ml. The amount of uredospores lodged on plants were approximately 2, 10, 20, 100, or 200 mg, respectively.

Estimates of uredospore production.-Ten fresh pustules formed on leaves after inoculation were cut at random 6 days after pustule formation, and then, every 3 days thereafter until the pustules were too old to produce spores. After every cutting, the spores in the remainning pustules on the leaves were removed entirely to permit new growth of uredospores and to prevent from the secondary infection. Each pustule on excised leaf were put on a slide with a drop of sterile water containing 0.1% wetting agent (Tween 80), and aggitated by a glass rod to free the uredospores from the pustule. The spores were counted under microscope by using a hemacytometer.

Results

Field screenings.-Peanut rust was severe at late growing season of the crop in 1979. Among the 461 accessions tested, only P. I. 314817, and P. I. 259747 showed less infection (fig. 1), and only P. I. 315608 showed no pustules on leaves while accession TN-4 was highly susceptible and then was chosen as a susceptible check. Rust symptom were observed 60 days after seeding and came to the log phase around 70 days. Two peaks of uredospore concentrations in the field were observed in July and December (fig. 2). The peaks appearance coincided with the later stages of the two peanut crops (spring and autumn crops) a year in Taiwan.

Rust nursery screenings.-Uredospores in the air 30 cm above the canopy in the rust nursery from May to December in 1979 were successfully maintained at 200 to 700 uredospores/3.24 cm^2/day (fig. 3). Accessions P. I. 314817 , P. I. 259747 and P. I. 315608 were tested in the rust nursery. The first two accessions showed different reactions among plants with most plants fully susceptible but few resistant or moderately resistant (fig. 4). The disease started 29 days after seeding and the disease progress curve of susceptible type came to the log phase around 70 days after seeding. The third

accession (P. I. 315608) again was immune or nearly so.

Greenhouse tests.-Peanuts of P. I. 314817, P. I. 259747 and TN-4 were susceptible, in terms of number of pustule , when inoculated with 20 mg uredospores/plant, regardless of plants ages (30, 60 or 90 days old), in the greenhouse. However, P. I. 314817 and P. I. 259747 had fewer, smaller pustules and produced less spores compared with TN-4 (fig. 5), and P. I. 315608 showed immune reaction at this inoculum density. With regard to the reactions of the four accessions to fourteen rust isolates collected from different peanut growing areas in Taiwan, no differential reactions were observed. The results of further tests indicated that accessions showed different degree of infection with different inoculum concentrations (fig. 6). Plants of TN-4 were very susceptible to rust fungus even at 2 mg of uredospores applied per plant, the lowest inoculum density tested(fig. 6). In contrast, plants of P. I. 259747 and P. I. 314817 showed complete susceptibility to rust only at the highest inoculum density (200mg uredospores per plant) and showed progressively less disease with each increment redution of inoculum density down to 2 mg uredospores per plant which resulted no disease (fig. 6). Plants of P. I. 315608 only showed some rust when 100 mg and 200 mg of uredospore inoculum was applied per plant.

Discussion

Accessions P. I. 314817 and P. I. 259747 expressed a slow rusting reaction and P. I. 315608 showed highly resistant or near immune to *P. arachidis* under natural environments in Taiwan. In rust nursery, where the inoculum density was higher than that in the natural field, accessions P. I. 314817 and P. I. 259747 became susceptible but accession P. I. 315608 remained highly resistant or near immune. In greenhouse tests with increasing amounts of inoculum, P. I. 314817 and P. I. 259747 showed increasingly more rust severity as the inoculum density was increased. P. I. 315608 was essentially immune in the greenhouse tests except when plants were inoculated with very high spore concentration (200 mg of uredospores per plant) plus a wetting agent (Tween 80) which produced heavy infection on other accessions as well. The accessions P. I. 314817 and P. I. 259747 showed a mixture of resistance may indicate that those two accession are not genetically uniform.

Van der Plank (1968) visualized horizontal resistance as a type that slows an epidemic after a disease has started. The resistance of P. I. 314817, P. I. 259747, and P. I. 315608 may be considered as horizontal, with P. I. 315608 having the highest form of resistance. The resistance of all three accessions could be overcome by heavy load of inoculum with the greatest load required to overcome the resistance of P. I. 315608 (fig. 6), Inoculum density plays an important role in the degree of infection and symptom expression.

No matter how soon or how late the disease started in the disease nursery (fig. 4) or under natural conditions in the field (fig. 2), the disease progress curves came to the log phase around 70 days after seeding. This indicates that the crop canopy and inoculum density at that time created a favorable enviroment for the disease epidemic.

No different physiological races could be identified based on the reaction types of

the fourteen local isolates of *P. arachidis* in Taiwan on the accessions P. I. 314817, P. I. 259747, P. I. 315608 and TN-4. These four accessions showed a similar levels of resistance to all isolates. The results were in agreement with those of Bromfield and Cevarios' (1970) who used two isolates from Texas and Puerto Rico. In our studies, P. I. 315608 showed a much higher levels of resistance than reported by McVey (1965), Cook (1972) and Romero (reviewed in Hammons, 1977), suggesting that either these workers may used a very high inoculum density in their experiments or that they may have a different physiological race.

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接種源濃度影響落花生對銹病菌之抵抗性[1]

林 益 昇[2]

摘 要

本研究篩選461個花生品系對銹病菌之抵抗性，僅 P.I. 314817, P.I. 259747, 及 P.I. 315608 三個品系在天然環境下表現緩銹現象（slow rusting），當這三個品系在擁有較天然環境更高量接種源之銹病圃篩選時 P.I. 314817及 P.I. 259747 則變爲罹病反應，而 P.I. 315608 仍是抗病反應。又有溫室中以胞子懸浮液加上展著劑（Tween 80）進行接種（200 mg胞子量／植株），則以上三個品系皆爲罹病反應。另外在溫室中以不同濃度(2～200 mg胞子量／植株)之胞子懸浮液噴到 90 天株齡的植株上時各品系皆表現不同程度之感染，而且夏胞子堆之產量亦有相當差距。可見銹病菌接種源濃度對各花生品系在感染之數量與病徵之表現扮演着重要角色。又從臺灣各花生栽培區收集的十四個銹病菌菌株，接種在 P.I. 314817, P.I. 259747, P.I. 315608 及 TN-4 四個鑑別品種上，並不能從其病徵之表現鑑別各菌株間有生理小種之分化現象。

1. 臺灣省農業試驗所 研究報告 第 957 號。

2. 本所研究員兼植物病理系系主任。臺灣省 臺中縣 霧峰鄉。

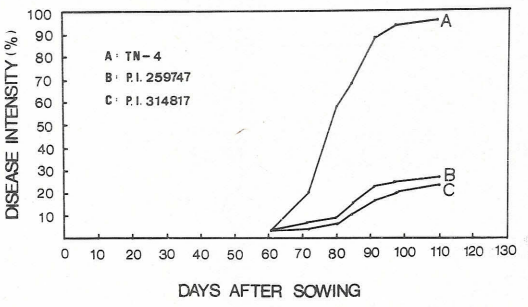


Fig. 1. Development of peanut rust on accessions TN-4, P. I. 314817, and P. I. 259747 under natural conditions in a field

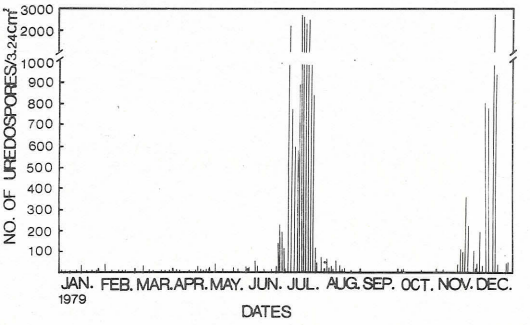


Fig. 2. Number of uredospores of *Puccinia arachidis* in the air 30 cm above a peanut field.

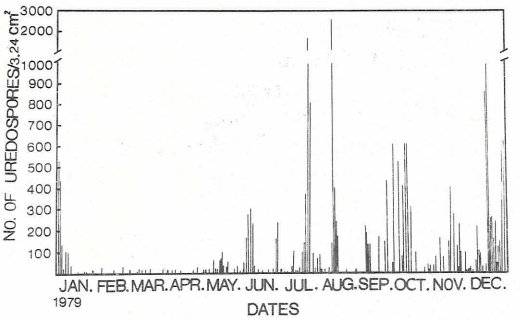


Fig. 3. Number of uredospores of *Puccinia arachidis* in the air 30 cm above a rust nursery, where inoculum was generated by continual planting of susceptible plants.

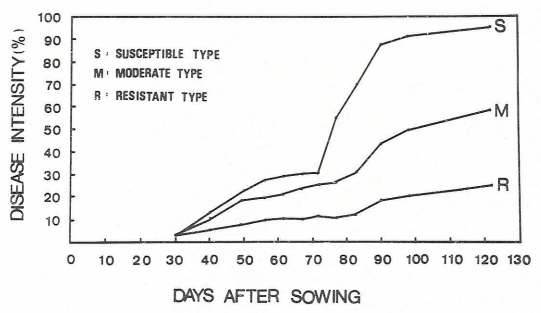


Fig. 4. Three types of peanut rust development among plants of accession P. I. 314817, and P. I. 259747 tested in rust nursery.

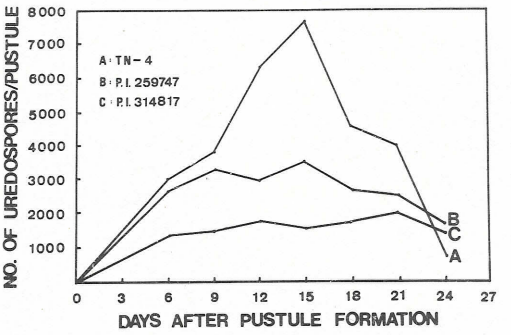


Fig. 5. Number of uredospores per pustule of *Puccinia arachidis* on accessions TN-4, P. I. 314817, and P. I. 259747.

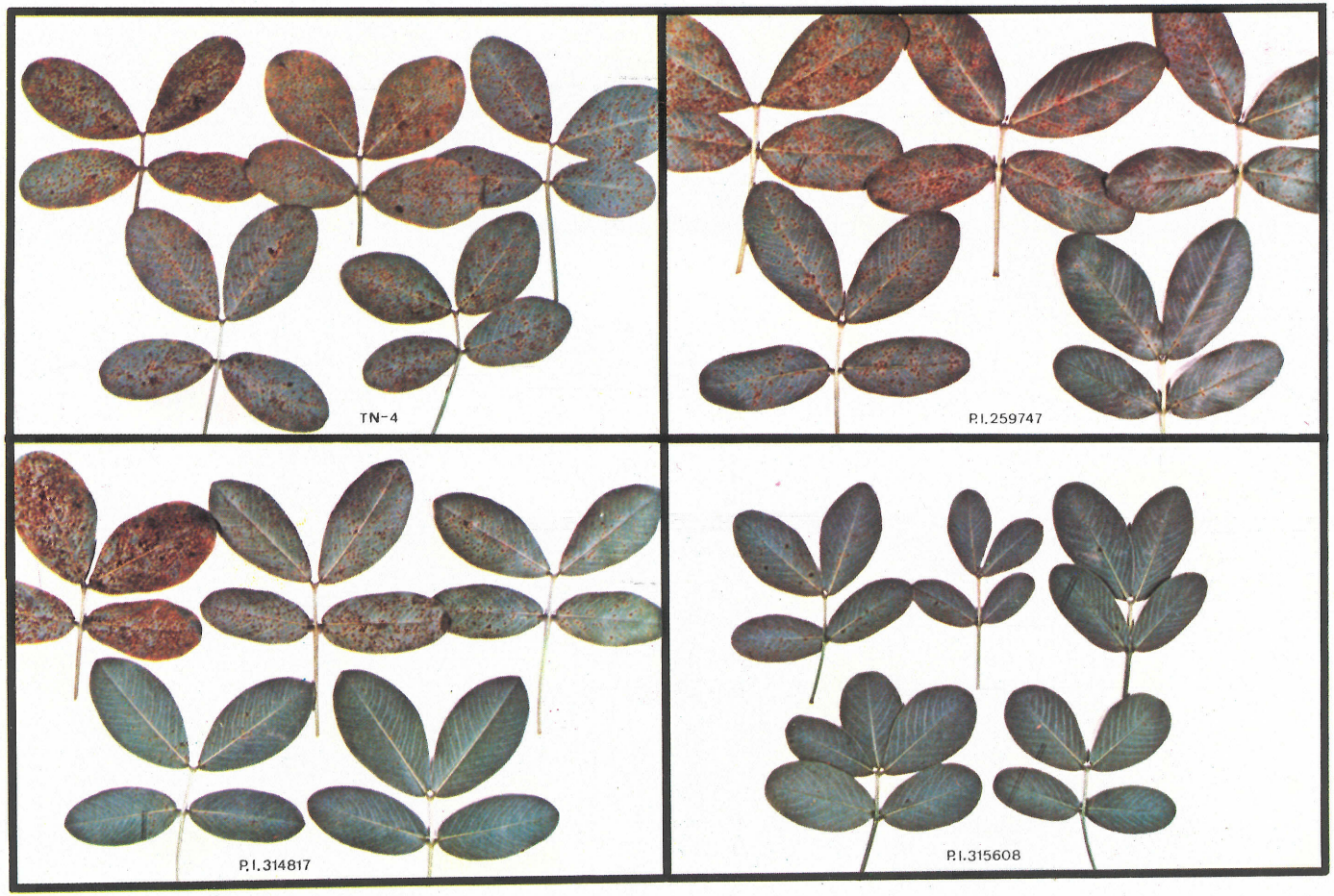


Fig. 6. Reactions of four accessions of peanuts to *Puccinia arachidis* when inoculated with different inoculum concentrations in greenhouse. Identify the treatments on each accession from left to right : (Top row) 200, 100, 20, (Bottom row)10, and 2 mg of uredospores per plant.