RESISTANCE TO ROOT ROT CAUSED BY *PHYTOPHTORA PALMIVORA* BUTL. IN *CARICA PAPAYA* L. : SCREENING, HERITABILITY, AND ASSESSMENT OF GROWTH UNDER NURSERY AND FIELD CONDITIONS

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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By

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by Raul Mosqueda-Vazquez

A dissertation submitted to the Graduate Division of the University of Hawaii in partial fulfillment of the requirements for the degree of Doctor of Philosophy

ABSTRACT

Papaya production in Hawaii is limited by the "replant problem". These studies were initiated because root rot caused by *Phytophthora palmivora* Butl. is an important component of this problem. Root rot resistance has been identified in the field. It is known however, that breeding for resistance by planting continuously in infested fields has some drawbacks and has not been entirely effective to date.

A reliable method of uniform inoculation is needed to accelerate breeding for resistance. Information on inheritance of resistance is lacking. Papaya plants surviving artificial inoculations are stunted in growth and it is not known if they can reach the reproductive stage and yield progenies which could be selected to accumulate resistance.

A group of papaya lines was chosen which had shown resistance and susceptibility to root rot in previous studies.

An existing method of inoculation that makes use of small chambers where young cotyledonary stage seedlings are suspended in a zoospore suspension of the pathogen was tested in a series of experiments. The

method was discarded because results failed to correlate well with field results.

A new method of screening for resistance was therefore developed using the P170 isolate of P. palmivora as inoculum. This method consisted of growing papaya seedlings in pots with vermiculite in a greenhouse for one month. A zoospore suspension of the desired concentration was poured into the pots. Mortality counts and disease rating of the seedlings were taken one month after inoculation. Survivors were then transplanted into the field.

By this inoculation procedure it was seen that: (1) developmental resistance in papaya begins to appear after the second week after germination; (2) in one week old seedlings of 'Higgins', a concentration as low as 200 sporangia/plant produced 50% mortality; (3) developmental resistance increased as seedlings became older and apparently was totally developed by the time seedlings were 2 months old; (4) comparisons among papaya lines inoculated with increasing concentrations of inoculum demonstrated that resistance exists; (5) papaya lines one month old, inoculated with a uniform concentration of inoculum, could be separated in three groups: resistant: Line 8, 'Waimanalo'-23, 'Waimanalo'-24 and Line 40; moderately resistant: 45-T_{22} and 'Kapoho'; and susceptible: 'Higgins'. The correlation between greenhouse and field mortality was 0.9355, which was highly significant and supports the reliability of the method.

A 5 x 5 half diallel crossing system was used to estimate heritability. All progenies were inoculated in the greenhouse by the described procedure. Although the two methods of statistical analyses used gave different values, both showed agreement on the presence of a

highly significant additive genetic variation for root rot resistance in the population studied.

All inoculated papaya lines were severely defoliated following inoculation in the nursery, but significant differences between lines were observed in plant height and stem diameter. The growth of Line 8 was not affected by the pathogen.

When transplanted into an infested field, resistant lines 'Waimanalo'-23 and 'Waimanalo'-24 recovered in growth 3 months and 4 months respectively after transplanting. No yield data were obtained but it was observed that surviving seedlings were able to produce mature fruit from which seed could be obtained to grow progenies for further selection.

A breeding program to introduce resistance into the current papaya cultivar used in Hawaii is proposed. This would begin with back-crossing to incorporate resistance, followed by phenotypic recurrent selection to accumulate resistance while keeping variability. Elite resistant plants could then be selected out of variable progenies. These would then be inbred to produce homozygous lines for use as varieties.

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INTRODUCTION

Plant diseases have been a major restriction to commercial crop production since ancient times. Papaya, an important fruit crop in Hawaii and elsewhere in the tropics is no exception. It is attacked by many diseases, but one of the most important ones is root rot caused by Phytophthora palmivora Butl., (Nakasone and Aragaki, 1973).

The disease, first discovered on the Island of Oahu in the late 1930's (Parris, 1939), has spread to the Island of Hawaii which today is the major papaya producing area in the State of Hawaii. By 1971 over 4,000 acres, previously under papaya cultivation, had been abandoned because of root rot problems (Ko, 1971).

Tolerance to the disease has been detected in some papaya lines planted in a "replant" field located at the Waimanalo Research Station (Nakasone and Aragaki, 1973). A breeding program using these materials began soon after the detection of tolerance in an effort to accumulate tolerance, but the behavior of progenies of selected lines in subsequent plantings in the same field has been variable. The variation has been attributed to heterogeneous distribution and concentration of the pathogen in the field, as well as variation in environmental factors from one planting season to the next.

The relationship between juvenility and susceptibility of papaya plants to this disease (Ko, 1971; Nakasone and Aragaki, 1973; Ramirez and Mitchell, 1975), suggests the possibility of using existing methods or developing of new methods of screening for resistance at early stages of growth in the nursery. Such a method would have to be based on controlled inoculations, including homogeneous concentrations of the

pathogen, and would have to be carried out under more uniform environmental conditions than those found in the field.

On the one hand, the method used to screen for resistance must be reliable enough to allow screening of large populations with minimum space requirements, thus saving labor costs of field plantings and accelerating the breeding program. On the other hand, controlled inoculations should permit the study of heritability of resistance to the disease. By using the proper genetic design, this parameter can be estimated so that it would be useful either in choosing the best breeding method to be used or in predicting genetic advance that could be expected under selection in case heritability was high.

The objectives of this research are: (1) to confirm the reliability of the existing method for screening or to develop one; (2) to determine heritability of resistance to Phytophthora root rot in papaya using controlled inoculations in the greenhouse; (3) to establish the degree of growth reduction caused by the disease compared to standard plants; and (4) to follow up the performance of inoculated survivors in the field and compare this with the performance of similar uninoculated materials growing under the same field conditions.

LITERATURE REVIEW

The pathogen

Phytophthora palmivora Butl. is a Phycomycete widely distributed in the tropics. It has been reported to attack a wide range of cultivated plants. It has been most thoroughly studied in regard to its effects on cacao although many other economically important tropical crops are affected in different ways by this fungus.

Chee (1974) reports as many as 139 plant species as hosts of *P. palmivora*. Among the crops attacked by this fungus he lists fruit and nut crops (pineapple, papaya, citrus, durian, nutmeg, avocado, cashew nut, betel nut), vegetables (tomato, onion, chili, eggplant, beans, peas), oil crops (coconut, oil palm, castor bean), other plantation crops (cotton, *Hevea* rubber, tobacco, black pepper, cacao), and a large number of ornamental plants. Most of these crops are usually grown in warm tropical areas where there is heavy rainfall.

The pathogen attacks a variety of plant parts, causing damping-off of seedlings, trunk canker, leaf and petiole necrosis, seedling blight, and root, fruit, bud, flower and calyx rots.

Waterhouse (1974) classified *Phytophthora palmivora*, based on sporangial morphology, as belonging to two typical forms and an atypical form. She also pointed out that within each of these morphological forms, two compatibility mating types exist. The most useful feature in distinguishing between typical form 1 and typical form 2 is the ratio of length to breadth of sporangia. Form 1 shows a range of ratios between 1.6 and 2.0 or more, whereas form 2 has a range of ratios between 1.3 and 1.4 and rarely exceeds 1.6.

According to Zentmyer et al. (1973) the development of new strains of P. palmivora is more likely to occur in areas where, isolates belonging into both A1 and A2 mating types are found. Both mating types have been found in almost all areas of the world. They studied 206 isolates and found 56% A2, 39% A1 and 5% that could not be classified. One of these isolates obtained from papaya in Hawaii was identified as A1 type; however other papaya isolates from Brazil, Australia, Saipan, and Sri Lanka were found to be A2 types. An isolate from Vanda orchid obtained in Hawaii was found to be of the A2 type.

Doo (1964), studying P. palmivora under laboratory conditions, found that it grows best at 28°C. He also found that a single zoospore can cause infection of leaves and fruits, but the disease develops more rapidly as more zoospores are present. Sporangia were produced 72 hours after inoculation.

Aragaki et al. (1967) found in laboratory studies, using a papaya isolate from Hawaii Island, that sporangia of P. palmivora require only water to germinate indirectly (producing zoospores), whereas in papaya fruit extracts, direct germination (formation of germ tubes) takes place. Temperature is another important factor, the optimum temperature for zoospore formation is between 20°C and 28°C.

Host-pathogen relationships and the papaya root rot problem

Parris (1939) first reported the appearance of a new disease attacking papaya plants in Hawaii. In 1941, Parris stated that this disease could damage the stem, fruit and roots, and it could become important in the future. He also pointed out later (1942) that 40% of the trees were affected in Kahaluu and Kailua, Oahu and of these 25%

were killed at the apex. Symptoms of root rot included: (1) retardation of growth, (2) leaves of a light green or yellow color which fall prematurely, (3) apex of the stem deteriorating in growth, and (4) the few living roots becoming discolored and partially decayed. Trees attacked by this fungus usually died. At that time Parris identified the causal pathogen of the stem and fruit rot as P. parasitica Dast., but considered that Pythium aphanidermatum was the primary cause of root rot, although he mentioned that wounded roots are subject to invasion by Phytophthora.

After detailed studies, Tokunaga and Bartnicki-Garcia (1971) reclassified what had been previously reported in Hawaii as P. parasitica Dastur by several authors as P. palmivora. In this research the root rot studied is attributed to Phytophthora palmivora Butl.

Trujillo and Hine (1964) considered both fungi P. palmivora and Pythium aphanidermatum to be causal agents of root rot. They reported that a severe attack produces rapid wilting of the plants and soft rot of the tap root, affecting vigor of the papaya trees.

Murashige et al. (1964) reported that papaya tissues buried in the soil produced growth reduction and caused mortality of papaya seedlings planted successively in the same field. Both fungi, P. palmivora and P. aphanidermatum were identified from unthrifty papaya seedlings in the field. They suggested that an interaction of the fungi with papaya residues might be releasing toxic substances which could be the cause of the disease.

Trujillo and Hine (1965) were able to isolate both fungi, P. palmivora and P. aphanidermatum from unthrifty papaya seedlings. They observed that papaya residues buried in the soil were colonized by both

pathogens in 48 hours. They also found a relationship between soil temperature, residue colonization and disease development. The optimum soil temperatures for development of the organisms were 30°C for Phytophthora and 36°C for Pythium. Further study of both fungi (Hine et al., 1965), has shown that their sporangia do not germinate when placed in papain. Zoospores were killed by papain concentrations as low as 10 to 20 ppm.

Marcley (1967) found that P. palmivora survived in soil either as chlamydospores or as resting zoosporangia. Greater numbers of chlamydospores were counted. He pointed out that the fungus could survive for more than 6 months in soil without host at temperatures ranging from 16°C to 34°C. The pathogen was recovered from soil with a moisture content as low as 10%. It was also recovered from soil subjected to flooding for 5 months.

Kliejunas and Ko (1974) demonstrated that the mortality of papaya seedlings was higher when inoculated with motile zoospores of P. palmivora than with non-motile zoospores. Papaya seedlings, planted in 2 liter containers filled with soil, were grown for about 2 weeks after germination and then inoculated with varying concentrations of zoospores. Mortality varied from 20% when the concentration of motile zoospores was 9×10^3 per container to 94% when concentration was 9×10^5 per container.

Further work carried out by Ko and Chan (1974) showed that sporangia of P. palmivora used as inoculum were more effective in producing infection of papaya seedlings growing in soil than either zoospores or chlamydospores. Using a concentration of 24×10^3 propagules per container, they obtained 16% mortality of papaya

seedlings inoculated with motile zoospores, whereas 49% and 98% mortality resulted when the inoculum was chlamydospores and sporangia respectively. The same comparison between propagules was made in assessing their colonization potential on papaya stems. The order of effectiveness was as follows: sporangia more effective than chlamydospores and zoospores which were equal between them. They estimated that one sporangium released an average of 16 zoospores.

Hunter and Kunimoto (1974) working on the Island of Hawaii confirmed that P. palmivora sporangia are effectively released and dispersed by rain-splash and wind-blown rain. They demonstrated that sporangia survive only a few minutes at relative humidities less than 100%, except that when they are attached to mycelial mats they are able to survive drying conditions for several hours during day time.

Ramirez and Mitchell (1975) working in Florida studied inoculum concentration of chlamydospores and zoospores of P. palmivora in relation to infection of papaya seedlings grown in soil. They found the percentage of infection higher at all levels of inoculum tested (1 to 250 chlamydospores/g of soil) with 75 days old plants than with 45 days old plants. Percent mortality was, however, higher in younger than in older plants. This last result confirms Ko's results in Hawaii (1971). Using zoospores as source of inoculum they found the same relationship. The level of inoculum necessary to produce 50% infection was 1.25×10^4 zoospores per plant compared to only 0.09 to 0.11 chlamydospores/g of soil required to cause the same level of infection. Mortality was first observed at a level of 10^4 zoospores per plant in 45 day-old seedlings.

Histological studies of root rot infection in papaya have not been

published, although a reference to cacao has been made by Asare-Nyako and Dakwa (1974). They report that in studies conducted by Asare-Nyako and Amponsah (unpublished) four to twelve days old cacao seedlings were infected in the area between the root tip and hypocotyl. They indicate that the fungus penetrates the epidermis and invades the phloem but occasionally, the secondary roots may be penetrated first with the fungus moving through the phloem toward the tap root region. The fungus continues growing into the ray cells to invade the pith. In some instances the root may become girdled as a result of lateral spread of the fungus. When this occurs above the secondary root region, the seedling collapses suddenly, showing typical wilt symptoms.

In Pinus radiata, Newhook (1961) has reported a non-lethal infection of roots caused by Phytophthora supp. Artificial inoculations were made using zoospores in aerated culture solution. Six weeks after inoculation many primary and secondary roots were dead but new roots were produced, even through infected cortical tissues. Twelve weeks after inoculation, only a few new roots emerged with reduction in growth of the aerial portion of the plants. In infected roots, mycelia were confined to the cortex and did not penetrate the endodermis, whereas in dead roots mycelia had invaded the endodermis.

Kanlong and Hendrix (1977) inoculated several plant crops with different isolates of Phytophthora species. They failed to find any relationship between ability of isolates to parasitize and kill plants, and ability to stunt growth of plants transplanted into inoculated soil. They suggested that in spite of the fact that Phytophthora has been considered a plant killer, the genus apparently possesses mechanisms of pathogenesis not dependent on parasitism.

The papaya 'replant' problem in Hawaii has been partially attributed to P. palmivora, and various proposals have been advanced to control the root rot disease. Most of these suggested solutions have been costly. In regard to the use of chemicals, for instance, Hamilton (1956) found that when soil was treated with Crag fungicide 974 (Mylone) the papaya plants were more vigorous and healthier than those growing in the same field but without fungicide. Murashige and Nakano (1965) also tested the soil fumigant Mylone combined with papaya replants at 0, 3, 6, 9, and 12 months after removal of the previous papaya planting. Best results were obtained when replanting was done at least 6 months after removal of the previous crop and combined with soil fumigation. They also discussed the possible role of toxic substances arising from papaya residues. Lange (1960) also reported that different chemicals used as soil fumigants were effective in maintaining plant vigor.

Recently, Ko (1971) proposed the use of "virgin soil" (soil obtained from an area where papaya has never been cultivated) placed in each hole of a new papaya planting located in a replant field. This technique is based on results showing that seedlings up to 3 months old are susceptible to the fungus but older plants are not.

Dealing with another aspect of the problem, Nakasone and Aragaki (1973) detected tolerance to root rot in certain papaya lines and hybrids. Such tolerance was rated as percentage of juvenile mortality, vigor ratings (using a scale from 1 to 5), and number of selectable trees in the respective populations. They indicated that Line 8 Solo, Line 44 B, and 'Waimanalo' were more tolerant than other lines. However, they observed a wide variation in response to three cycles of selection. This could be due to heterogeneous distribution and

concentration of the pathogen in the field, or to variation in environmental factors from one planting season to the next.

Kinds of disease resistance

Nelson (1973) considered that there are two kinds of disease resistance in plants: (1) "the host resists the establishment of a successful parasitic relationship by restricting the infection site and the infection process", a type of resistance referred to as hypersensitivity, specific resistance, non-uniform resistance, vertical resistance or major gene resistance; (2) "the host resists the colonization and growth of the parasite, subsequent to a successful infection, even though the infection process, culminated by reproduction of the parasite is completed", a type of resistance referred to as field resistance, generalized resistance, non-specific resistance, partial resistance, uniform resistance, horizontal resistance, multigenic resistance, polygenic resistance, and minor gene resistance.

Van Der Plank (1968) considers that vertical resistance is present in a host variety when such a variety is more resistant to some races of a pathogen than to other races. In other words vertical resistance always involves interaction between varieties of the host and races of the pathogen. When this vertical resistance is present in the host, the pathogen is considered to have races which differ in virulence.

On the other hand, horizontal resistance is present in a variety of the host when its response to different races of a pathogen is similar in level of resistance. In other words there is no interaction between varieties of the host and races of the pathogen. When this happens, it is said that races of the pathogen differ in aggressiveness.

Aggressiveness has been considered to be partially dependent upon: "ability to produce large amounts of viable inoculum and the speed with which a fungus can penetrate and infect the host", (Knutson and Eide, 1961). They further stated that in order to have comparable quantitative estimation of field resistance in a host as well as degree of aggressiveness in a pathogen, each individual of the host should be inoculated with the same amount of inoculum.

Hooker and Saxena (1971) classified inheritance of disease resistance into three classes comprised of several types:

1. Oligogenic or Mendelian inheritance.

(a) Single genes. Most commonly, dominant genes are found conferring the resistance. This type of resistance is usually stable under very different environments.

(b) Reversal of dominance. In some cases a gene shows dominance for resistance to one race but acts as a recessive to different races of the same pathogen.

(c) Allelic series. Several genes located in the same locus confer resistance to different races of the same pathogen.

(d) Two or more genes. Two to six dominant genes acting independently have been identified as causing resistance to the same pathogen in one host.

(e) Gene interaction. Complementation, modification and epistasis have been found in many cases.

(f) Modifier genes. Sometimes a host has a major gene for resistance but its expression could be partially or totally inhibited by another gene. In other cases the modifier can enhance resistance.

2. Polygenic inheritance.

This type of inheritance has been detected in many cases. Progenies of resistant by susceptible crosses show continuous distribution with a tendency to produce a normal curve. Most studies show that additive genetic variation is the most important component of genetic variance and therefore genetic improvement could be attained relatively rapidly.

The possibility of the presence of both oligogenic and polygenic systems of inheritance in any single host cannot be excluded, (Van Der Plank, 1968; Hooker and Saxena, 1971).

3. Extrachromosomal inheritance.

This has been shown to exist in plants, although rarely found.

Before the 1970's considerable attention was devoted to the study and use of vertical resistance in crops such as cereals, flax and others. Success was achieved particularly in the cereal crops, resulting in many resistant varieties. Lately, a shift in trend is occurring from the knowledge that horizontal or polygenically inherited resistance would not be overcome as easily as the vertical resistance or oligogenically inherited type that is nullified by the appearance of new races of the pathogen, (Nelson, 1973).

Because of the fact that horizontal resistance is usually polygenically inherited, studies on heritability determination and type of gene action involved have become the new aim of plant breeders dealing with disease resistance, (Simons, 1972).

Breeding for disease resistance, rating scales and inoculation techniques

Verhalen et al. (1971), studying resistance to Verticillium wilt

in cotton, found that such resistance has heritability values up to 64%, which in their opinion, makes it easy to use simple selection to improve the studied population. They used the half diallel design for their study. Similar results were obtained by Nelson and Scott (1973) on resistance to corn stunt, a virus disease. They indicated that additive gene action was more important than non-additive, and proposed the use of recurrent selection to improve this corn population. They also made use of the half diallel design.

Using other methods available to determine heritability, Smith and Ruppel (1974) concluded that significant additive gene action is involved in determining leaf spot resistance in sugarbeet. Heritability reached values up to 26.7%.

Guzman-N (1964), studying several species of Solanum for partial resistance to Phytophthora infestans, compared the effectiveness of selection between seedling populations selected in the field and in the greenhouse. Selection in the greenhouse population was done by inoculating seedlings at early stages of growth. Inoculations were done with sprays of zoospore suspensions up to 25,000/ml. Disease development was rated five days later. Susceptible plants were discarded and the remaining plants sprayed with Maneb to inhibit spread of the disease. The progenies from the top 10% of the plants in both, greenhouse and field populations which showed resistance were then planted in the field and rated for disease resistance. The results showed an equal effectiveness with both methods, indicating the advantage of the greenhouse method for breeding programs inasmuch as larger populations can be screened for resistance at lower cost.

Lockwood and Williams (1957) studied inoculation and rating methods

for bacterial wilt of sweet corn. They used the three youngest fully expanded leaves for disease ratings. Their scale was from 0 for a healthy leaf to 5 for a dead leaf. They also used amount of wilting and severity and area of one leaf-lesion.

Vakili (1965), studying Fusarium wilt resistance in plants of Musa spp., reported that different sources of seedlings inoculated first by immersing their roots in microconidial suspensions of the fungus during a night, then again one month later by infesting the soil, and then transplanted 3 months later into infested soil, showed different mortality rates. Vakili considered that such differences were genetically determined.

Acosta et al. (1964) used not only the percent of survivors to rate resistance to bacterial wilt in tomato, but also a coefficient termed, "number of days the plants lived in infested soil".

Nelson and Scott (1973), studying resistance to corn stunt, used a scale from 1 for healthy plants to 6 for plants stunted 50% or more or dead.

Verhalen et al. (1971) rated cotton materials inoculated at seedling stages and under field conditions in their response to Verticillium wilt with a scale from 1 for no visible leaf symptoms or vascular discoloration in stems, to 10 for defoliated stems, dead down to ground level. They realized and emphasized that their conclusions were based largely on their rating system, and because of the subjectivity of ratings they warned other researchers about this. They emphasized the need for a standard system of rating the degree of infection for each particular disease.

Zentmyer and Mircetich (1965) pointed out that Phytophthora and

other fungi can infect plants growing in nutrient solution because they produce zoospores. In their screening procedure, they classified avocado seedlings into 10 groups 12 days after inoculation according to the severity of damage to the roots. They discarded those seedlings in the last two classes (81% to 100% of roots rotted), but kept the others for further observation. Further studies (Khew and Zentmyer, 1973) demonstrated that zoospores of P. palmivora have higher chemosensitivity to four aminoacids (arginine, aspartic acid, glutamic acid and methionine) than zoospores of P. cinnamomi and P. citrophthora.

In alfalfa, the inheritance of resistance to root rot caused by P. megasperma Drechs. has been studied by Lu et al. (1973). The genetic materials were transplanted in infested sand in water-tight tanks. The plants were dug after four weeks of flooding, and were rated on a scale of 1 to 5, one for no visible root lesions and 5 for more than 1/3 of the taproot rotted.

For rating Phytophthora root rot on papaya, Trujillo and Hine (1964) used a scale of 0 to 5, with 0 for healthy plants and 5 for dead plants. They observed that the vigor of plants attacked by the fungus was greatly reduced.

As mentioned previously, Nakasone and Aragaki (1973) used a scale to rate vigor of papaya plants in a breeding program for resistance to root rot. This scale was from 1 to 5, with 1 being low in vigor and 5 for vigorous. They also used seedling mortality. This variable was also used by Ko (1971), and Ramirez and Mitchell (1975). The percent of selectable plants is another variable used by Nakasone and Aragaki (1973), and is defined as the percentage of plants with vigor ratings greater than 4.

Turner and Asomaning (1962) first reported root rot on cacao to be caused by P. palmivora. Cacao seedlings growing in washed sand and sterilized soil with mature first flush leaves were inoculated by pouring a sporangial suspension on the surface of the soil. Variables used in computing differences were stem length, leaf number, leaf length, fresh and dry weights of leaves, stems and roots eight weeks after inoculation. They reported a reduction in absorbing area of roots as well as a decrease of 24% in stem length, 45% in leaf number, and 60% in leaf area. Fresh and dry weight reductions were also recorded and compared to those of uninoculated controls. The plants growing in sand were more severely affected than those in soil.

Using the same inoculation method described, Asomaning (1964) found a high correlation between root rot resistance and black pod resistance in several clones of cacao.

Zentmyer et al. (1968) tested two methods of inoculation of cacao clones which had different levels of field resistance to black pod with P. palmivora. They inoculated stems by making 5 mm length vertical cuts in the cortex of the stems and inserting 3 mm discs from PDA culture of P. palmivora and wrapping the wound with tape. Root inoculations were made by planting seedlings in individual containers with Hoagland's nutrient solution. One fourth of a petri dish culture of P. palmivora growing on V-8 juice agar was placed in cheese cloth and suspended in the nutrient solution. Stem cankers were rated by measuring lesion diameter 6 weeks after inoculation. Root rot estimates were made on percentage of decayed roots and by evaluating the condition of the top, from 0 = healthy to 3 = severely wilted recorded every week for 5 weeks. It was concluded that stem inoculations gave high correlations with

resistance to black pod, whereas root inoculations, while giving similar trends, did not show as pronounced a difference as stem inoculations.

In comparing pathogenicity of different isolates of P. palmivora, Zentmyer (1972) showed by using the stem canker test on cacao stems, that isolates from rubber and papaya plants had very low pathogenicity compared with an isolate from cacao. When papaya seedlings were inoculated on the stem with papaya, black pepper, rubber and cacao isolates, there were significant differences among isolates. Isolates from cacao showed low pathogenicity to papaya. It appears that isolates from different hosts and even isolates from cacao from different areas of the world have differences in pathogenicity.

Blaha (1974) indicated that in testing for resistance to P. palmivora in cacao, the method of inoculation should resemble the natural process. The isolate to be used should be highly virulent and representative of the area under study. He stressed that several authors have agreed that the use of local strains of the pathogen should be preferred over strains from different origin.

Amponsah et al. (1973) devised a method of screening seedlings for black pod resistance in cacao. Four or five day-old seedlings were immersed for 3 minutes in a sporangial suspension of P. palmivora and planted in sterilized soil. Twelve days later, emergent seedlings were counted. The survivors were counted eight weeks after inoculation and those appearing healthy were transplanted into larger containers for later field transplanting. A higher proportion of resistant seedlings was found in progenies of black pod resistant clones than in progenies of susceptible clones. Immune plants have not been found to date, and high inoculum concentrations can kill all inoculated plants.

Aragaki (1975) devised a technique for inoculating papaya seedlings, using zoospores of P. palmivora in a suspension in deionized water. Papaya seedlings were inoculated when the cotyledonary leaves were fully expanded. Sporangia grown 4 to 7 days in 10% V-8 juice agar were used for inoculation. He has used an inoculation time of 4 hours. At the end of this period seedlings are rinsed with a solution of 0.05% sodium hypochlorite for ten minutes and transplanted into individual 6 cm peat pots in vermiculite. The seedlings are observed for three weeks. The first week after inoculation was the most critical period. He reported 60% mortality in 'Sunrise' Solo papaya 17 days after a 2-hour inoculation period. He did not observe further kill after 17 days.

Some methods of studying heritability of characters

Genetic properties of a population can be determined on the basis of an analysis of variance, partitioning the phenotypic variance into heritable and non-heritable components. The data analyzed are the phenotypic measurements of any continuous trait shown by the individuals of the population (Falconer, 1960).

Several genetic designs have been devised, which enable the researcher to estimate genetic and environmental components, depending upon the mating system of the species studied. Data are obtained from different generations (parents, F_1's, F_2's, backcrosses, and selfed and intercrossed generations can be used). Among the usual genetic designs are the randomly mated biparental progenies, the North Carolina designs 1, 2, and 3, the triple test cross, and diallels. Diallels are well suited to hermaphrodite species, and the availability of true inbred

lines brings about many advantages in partitioning the genetic variance (Mather and Jinks, 1971).

The diallel cross as defined by Hayman (1954b) is a complete set of all possible matings between several genotypes. Genotypes are defined as individuals, clones or homozygous lines. He also defined a diallel table as an arrangement in a square of n^2 measurements corresponding one-to-one to the mating combinations of a diallel cross, each row and column of the square corresponding to offspring with a common parental genotype. Hayman (1954a, 1954b) considered a diallel cross to be composed of n selfed lines plus their n^2 - n crosses. Subsequently this design has been called "complete diallel" to distinguish it from modifications which have arisen.

The assumptions on which the complete diallel is based in order to be analyzed and simplified are (Hayman, 1954b): (1) Diploid segregation, (2) No differences between reciprocal crosses, (3) Independent action of non-allelic genes, (4) No multiple allelism, (5) Homozygous parents, (6) Genes independently distributed between the parents. The author has also provided the mean to test for failure to meet such assumptions.

Griffing (1956a) classifies the diallels into 4 experimental methods. Method 1 includes parents, F_1's and their reciprocals (this is the complete diallel). Method 2 only includes parents and one set of F_1's (this is the half diallel). The term "modified diallel" was introduced to mean his methods 3 and 4 of analysis. In method 3, the reciprocal F_1's are included but the parental lines are not, whereas in method 4, only one set of F_1's is grown but the parental lines are not. He demonstrated that his proposed analyses give estimates which are

equivalent to those obtained by the classic technique of covariance between parents and offspring.

Griffing's method of analysis (1956b) emphasizes the usefulness of specific and general combining ability estimates. In his methods 3 and 4 however, disregards the parental lines, and in Hayman's opinion (1960), thereby loses genetical information.

On the other hand, Hayman's analyses (1954a, 1954b, 1960) provide enough statistics to estimate five components of variation, namely D, F, H_1, H_2 and h^2. D measures additive effects, H_1, H_2 and h^2 measure dominance effects, and F provides an indication of whether or not dominant alleles are more frequent in the parents than recessive ones. Component E which is the environmental component of the total phenotypic variation, can also be estimated by using an appropriate experimental design with adequate replications. In addition using an additive-dominance model, when only parents and F_1's are available, heritability can be calculated both in the narrow sense and the broad sense (Mather and Jinks, 1971).

In Mather and Jinks' opinion (1971), the analysis proposed by Hayman for a complete diallel set of crosses is the best available. Sometimes, however, the researcher has limited funds or insufficient space in which to grow the reciprocal crosses. In some instances it is known that differences between reciprocal crosses are unlikely to occur. In such cases a half diallel is best suited since only the selfed parents and one set of F_1's are needed and the genetic information obtained is still adequate. Inferences regarding reciprocal and maternal effects are not possible in a half diallel. Jones (1965) has presented the analysis of variance for this modification, following the theory

proposed by Hayman already mentioned.

Kearsey (1965) working with a randomly breeding population experimentally compared five genetic designs: Biparental progenies, North Carolina designs 1, 2, the partial diallel (another modification of the original diallel, proposed by Kempthorne and Curnow), and the half diallel. He concluded that the half diallel yielded the largest amount of information about the components of variation, although it has the restriction of a small sampling power. Estimates of the components proved the most reliable of all of the designs tested. In the absence of maternal effects, the half diallel is preferred therefore over the complete diallel because it yields almost the same information with much less expenditure.

As has been mentioned above, the half diallel allows the determination of heritability. Since heritability is the ratio of additive genetic variance to phenotypic variance (narrow sense) or the ratio of genetic variance to phenotypic variance (broad sense), (Falconer, 1960), its value indicates the degree to which the trait under consideration is inherited. As a matter of fact, determination of heritability is critical to the breeder, as pointed out by Falconer (1960), because the choice of breeding method is dependent on the heritability value.

Mather and Jinks (1971) suggest that in dealing with quantitative inheritance the researcher must necessarily be aware of the scale used in measuring the character under study. They suggest that a change in scale may change the values and even lead to different genetic interpretation. They emphasize that the scale selected for genetic analysis must be the result of careful consideration.

MATERIALS AND METHODS

ASSESSMENT OF ROOT ROT RESISTANCE IN PAPAYA

Field study

Two fields, Q-1 and O-1 at the Waimanalo Research Station have been utilized since the early 1960's for repeated papaya plantings in a breeding program for Phytophthora root rot resistance. In June 1974 a experiment was established in field O-1 which included 27 papaya lines and sublines (individual selections within lines) and several hybrids. The experiment was set up in a randomized complete block design using 4 replicates. This planting was the 5th consecutive papaya planting in the same field. Previous papaya plantings in this field had plants damaged and stunted by root rot caused by P. palmivora. Infected plants which were not killed by the pathogen showed poor growth (Nakasone and Aragaki, 1973).

All of the breeding material included in the 1974 planting are strongly related genetically to the original 'Solo' type (Nakasone, 1975), introduced to Hawaii early in this century (HAES, 1920).

Plants were spaced 3.0 m between rows and 1.8 m between plants. Each hole was planted to two seedlings and females were rogued out as soon as they flowered and could be detected. There were 10 plants per experimental unit at the beginning of the experiment and 5 after roguing.

Mortality was recorded up to 3 months after transplanting. Disease ratings were taken the third month after transplanting, using a scale of 1 to 5, with a rating of 1 for dead plants and 5 for healthy, vigorous plants. The analysis of variance for the two variables was carried out accordingly. Percent mortality was transformed to arc sin

$\sqrt{\%}$ to stabilize the variances (Snedecor and Cochran, 1967). The K-ratio t, (LSD) test (Duncan, 1975) also called the Waller-Duncan's Bayesian K-ratio t or LSD rule (Chew, 1976) was used to test the significance among papaya line means. This test will be called K-ratio t in this paper.

Greenhouse experiments

Plant materials. Table 1 lists the papaya lines and cultivars used in this research together with their source and mating systems.

Papaya seedlings used for laboratory or greenhouse inoculations were germinated in community pots with No. 2 grade vermiculite used as germination media. Seeds were surface disinfested by a 5 minute-soak in a 0.05% sodium hypochlorite solution followed by a rinse with deionized water before sowing. After sowing, the seeds were watered once or twice daily depending upon prevailing temperatures in the greenhouse. Germination occurred between 12 and 20 days after sowing in most cases. Seedlings were fertilized by sprinkling a 0.3% solution of a commercial complete fertilizer with major elements 20-20-20 and minor elements (B, Mo, Mn, Mg, Fe, Cu, and Zn) over the plants at a rate of 30 ml/plant. Fertilizer was applied weekly, but suspended seven days prior to the inoculation date in experiments with plants more than 2 weeks old and resumed one week after inoculation date. Neither pests nor diseases were encountered in the greenhouse experiments. The test organism used in all experiments was isolate P170 of Phytophthora palmivora, described by Aragaki (1975).

Periodical isolations from roots of inoculated papaya seedlings were made and plated in water-agar to check the presence of the pathogen throughout the experiments.

TABLE 1. -- *Carica papaya* populations used in different experiments to assess resistance to *Phytophthora* root rot

Accession number	Name if a cultivar	Source	Mating system
17 A	Higgins	U. of Hawaii	Gynodioecious
25	Wilder	U. of Hawaii	Gynodioecious
40	------	U. of Hawaii	Gynodioecious
41	------	U. of Hawaii	Gynodioecious
$45\text{-}T_{22}$	------	U. of Hawaii	Gynodioecious
77[x]	Waimanalo	U. of Hawaii	Gynodioecious
63-22	Sunrise Solo	U. of Hawaii	Gynodioecious
Line 8	Line 8	U. of Hawaii	Gynodioecious[y]
26-2	Kapoho Solo	U. of Hawaii	Gynodioecious
72-8(71-4) T OP	------	U. of Hawaii	Gynodioecious
Line 83 OP	------	Thailand	Gynodioecious
Line 122 OP	------	Malaysia	Gynodioecious
HAES 8018	La Chola Roja	Panama	Gynodioecious[z]
HAES 8022	Hortus Gold	South Africa	Dioecious[y]
L-1-17	------	Mexico. INIA	Polygamous
L-3-6	------	Mexico. INIA	Polygamous
L-15-M	------	Mexico. INIA	Polygamous
L-15-14	------	Mexico. INIA	Polygamous
L-18-16	------	Mexico. INIA	Polygamous

[x] Two sublines were employed, 77-23 and 77-24.

[y] Mating system mentioned by Storey (1969).

[z] Personal communication (R. A. Hamilton, June, 1977).

A. Refining of Aragaki's laboratory-greenhouse assay method.

1. Determining optimum concentration of inoculum and time of exposure to cause a specific mortality percentage in a susceptible papaya line.

Aragaki (1975) used 'Sunrise' Solo which he had observed to show higher tolerance to root rot than certain other cultivars under field conditions. In the present study from the field evaluation described above, and from previous information (Nakasone and Aragaki, 1973), it was decided to use papaya cultivar Higgins as a test line since it had shown high susceptibility to root rot under field conditions.

Since concentration of inoculum and time of exposure to inoculum are factors influencing percent mortality in papaya seedlings, an experiment was planned to determine the optimum levels of both factors needed to produce 70% mortality in seedlings of 'Higgins', using Aragaki's method assay.

Nine treatments were included: 4 concentrations of inoculum (0, 250, 500 and 1,000 sporangia/ml), and two exposure times, 2 hours, and 4 hours. A randomized complete block design was set up using 4 replications. Twelve seedlings at the fully expanded cotyledonary leaf stage were used for each inoculation chamber (Fig. 1). The number of sporangia for each concentration was estimated by counting 10 fields of a hemacytometer immediately after sporangia were brushed off the petri dish into a known volume of deionized water.

Mortality counts were made daily starting on the third day after inoculation and terminating on the 30th day. Analysis of

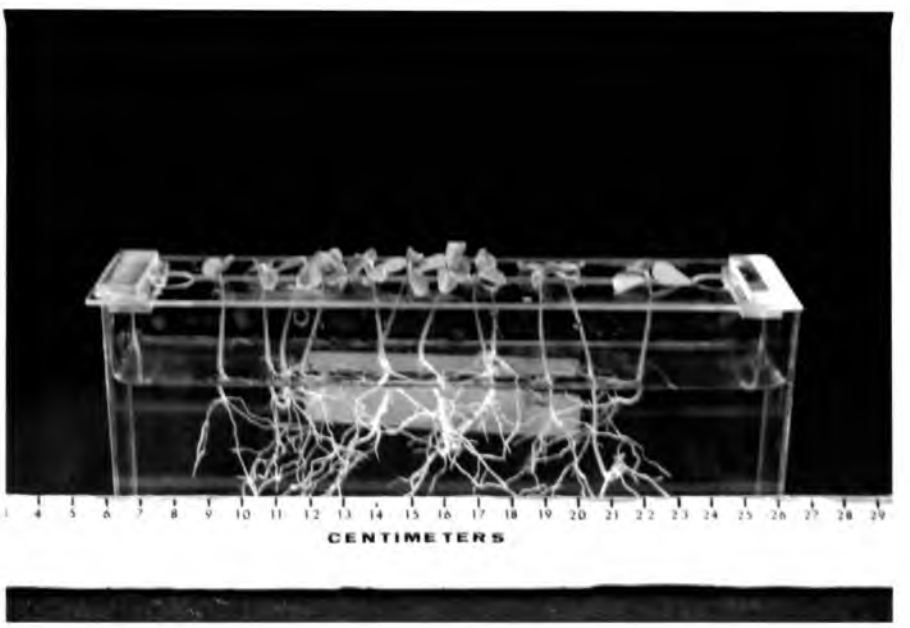


Fig. 1. Inoculation chamber, after Aragaki (1975)

variance of percent mortality was carried out. The sum of squares for treatments was partitioned into the following comparisons: controls vs. inoculated, 2-hour inoculated vs. 4-hour inoculated treatments, and linear regression of percent mortality on concentration of inoculum for each of the 2 and 4-hour inoculated treatments, as suggested by Chew (1976). The remaining 4 degrees of freedom corresponding to deviations from regression.

The usual regression model for a straight line $\hat{Y} = a + b\ X$ may not be appropriate in cases where zero inoculum causes no disease, resulting in zero mortality and the regression line does not pass through the point of origin. Calculations were therefore performed to reduce this model to $\hat{Y} = b\ X$ to fit the regression line through the point of origin. Prediction of X (concentration) from an assumed value of Y (% mortality) was done as indicated by Snedecor and Cochran, 1967.

2. Comparison of 7 papaya lines ('Solo' type) with 'Higgins' for response to inoculation by the laboratory-greenhouse method.

The papaya lines included in this experiment were: 'Waimanalo'-23, 'Waimanalo'-24, 'Sunrise' Solo, 'Higgins', 40, 41, 45-T_{22} and 25. Aragaki's inoculation method was employed with modifications in concentration of inoculum, and time of exposure to inoculum. These last two factors were the ones found in a previous experiment.

The experiment was set up as a completely randomized design with treatments replicated according to the number of seeds available. The smallest number of replicates was 4 and the largest was 7. Bartlett's test of homogeneity (Snedecor and Cochran, 1967) was applied to the estimated variances for each treatment prior to

analysis of variance. Percent mortality was the variable analyzed. The K-ratio t test was used to assess significance in comparing each line against 'Higgins' which became the check in most experiments. A correlation coefficient was also calculated between percent mortality of the 7 lines obtained for 4 continuous field plantings and percent mortality for the same lines which had been inoculated by the laboratory-greenhouse method.

3. Further comparison of response to laboratory-greenhouse inoculation at different levels of inoculum between line 40 and 'Higgins'.

Further testing was done using the same concentrations of inoculum (0, 250, 500, and 1,000 sporangia/ml) as in the very first experiment to show that the response of line 40 was similar to that of 'Higgins' under similar conditions. A two hours inoculation period was used. The experiment was completely randomized and data taken on percent mortality analyzed. A linear regression of percent mortality on concentrations of inoculum was done, and comparison between the regression equations for line 40 and 'Higgins' was made using the general linear test as indicated by Neter and Wasserman (1974).

4. Comparison of response to laboratory-greenhouse inoculation of papaya seedlings from foreign accessions with those of 'Higgins'.

An experiment was carried out to test the response of foreign papaya accessions compared with that of cv. Higgins to determine whether or not the method of inoculation used or the narrow germplasm base was responsible for the failure to detect

differences in response to Phytophthora inoculations.

Papaya seeds of the following populations were used in this experiment, Line 83 OP, Line 122 OP, HAES 8018, L-1-17, 1-3-6, L-15-M, L-15-14, L-18-16, 72-8(71-4)T OP, and 'Higgins'.

The experiment was carried out using Aragaki's method of inoculation with modifications introduced during the early part of this study. Two treatments were replicated 3 times, and 8 treatments were replicated 4 times. Analysis of variance was carried out on percent mortality, and the K-ratio t calculated to test significance between treatment means.

B. Testing a different method of inoculation.

Inoculation of papaya seedlings older than at fully expanded cotyledonary leaf stage was not possible using the method of assay devised by Aragaki (1975), because root damage during the handling process would be more detrimental to the older plants. Hydroponic culture can be used to inoculate older papaya plants as described for avocados (Zentmyer and Mircetich, 1965). There were no facilities available for this method and the number of seedlings which could be screened at any one time would be very limited with this system. A method of inoculation was therefore developed to meet the needs of these studies, which were to simultaneously screen large populations of seedlings in a small space, and using ordinary greenhouse facilities such as benches, watering, and protection from pests and other pathogens.

The method which is simple makes use of some steps in Aragaki's method (1975) as outlined below:

(1) Seedlings were germinated in community pots filled with No. 2

horticultural grade vermiculite. (2) About 20 days after planting the seed young seedlings were transplanted into individual 8 cm depth peat pots using the same type of vermiculite as the growing media and kept in the greenhouse. (3) For purposes of these studies the germination date for all seeds was considered to be the 16th day after sowing the seed although germination varied from 12 to 20 days. (4) Water and fertilizer were provided as indicated previously. (5) Cultures of the single zoospore isolate P170 of P. palmivora were grown on vegetable juice agar (10% Campbell's V-8 juice, 0.2% $CaCO_3$) in petri dishes under continuous Cool White fluorescent irradiation (approx. 2,700 lx) at 24°C for 4 to 6 days (Aragaki, 1975). (6) Sporangia from 1 to 3 petri dishes were brushed off into 100 ml of deionized water at 24°C. (7) Estimates of number of sporangia were made by counting 10 fields of a hemacytometer for every 100 ml of suspension, and calculating the concentration as sporangia/ml in the suspension. Dilutions were made as necessary on the basis of estimated number of sporangia in the original suspension. (8) When the seedlings were ready for inoculation they were irrigated at 7:30 a.m. and inoculations were made between 11:00 a.m. and 3:00 p.m. (9) Plants were inoculated with 24 ml of zoospore suspension from a dispenser poured directly into each pot, taking care not to spill the suspension on leaves or stems of the papaya seedlings (Fig. 2). (10) By this method about 600 seedlings could be inoculated by one person in 3 hours.

1. Comparing 4 levels of inoculum on seedlings of 'Higgins' at 4 different ages using the greenhouse inoculation method.

Potted seedlings of four different ages were inoculated in independent experiments to compare their response to different

Fig. 2. Pouring zoospore suspension into pots containing one-month old papaya seedlings

levels of inoculum. Two-week old seedlings were inoculated with 0, 1, 500, 3,000 and 4,500 sporangia/plant. One, 2, and 3-month old seedlings were inoculated with 4,500, 9,000 and 18,000 sporangia/plant with an uninoculated check.

Two replicates were used for all experiments with the exception of 2-month old seedlings, where three replicates were used. Mortality counts were taken weekly up to 1 month after inoculation. The linear regression method was used to analyze percent mortality on concentration of inoculum. The general linear test (Neter and Wasserman, 1974) was employed to compare regression equations obtained for each of the seedling ages tested.

2. Determining the minimum concentration of inoculum at which a significant response in mortality is obtained in 1-week old seedlings of 'Higgins'.

Five concentrations of sporangia/plant (8, 40, 200, 1,000 and 5,000) were compared against a control (0 sporangia/plant) inoculating 1-week old seedlings of 'Higgins' to determine the lowest concentration at which a significant mortality response could be obtained.

The experiment was set up as randomized complete block design, with two replicates. Percent mortality was recorded and the data transformed to arc sin $\sqrt{\%}$ (Snedecor and Cochran, 1967). Analysis of variance was performed. The lowest concentration at which significant mortality occurred was identified by using the $\bar{t}$ test in sequential form as indicated by Williams (1971).

3. Testing three inoculum levels on 1 and 2-week old seedlings of 'Higgins', 'Kapoho' Solo and 'Waimanalo'-23.

Two experiments were set up as split-plot design with sub-units in strips to compare differences in response to inoculation between seedlings of 'Higgins', 'Kapoho' Solo and 'Waimanalo'-23. These cvs. were allocated into the whole units and the levels of inoculum which were 0, 1,500, 3,000, and 4,500 sporangia/plant were allocated into the sub-units. Two replicates were used in each experiment. Each sub-unit was made up of 12 plants. One experiment was designed to test 1-week old seedlings, and the other one to test 2-week old seedlings. Since the degrees of freedom available to test significance between cvs. were low, regression techniques were used to calculate percent mortality on levels of inoculum for each cultivar and the general linear test (Neter and Wasserman, 1974) was used to compare the regression equations.

4. Testing three inoculum levels on 1 and 2-month old seedlings of several papaya lines.

In the first experiment, 10 papaya lines were included, 7 of the 'Solo' type (40, 41, 25, 72-8(71-4)T OP, 'Waimanalo'-23, 'Sunrise' Solo, and 'Higgins') and 3 of the long-fruited type (L-3-6, L-15-14, and HAES 8018).

In the second experiment with 2-month old seedlings the following 11 papaya lines were included: The seven 'Solo' listed above, line 45-T_{22}, the three long fruited types (L-3-6, L-15-14) and HAES 8022 instead of HAES 8018.

In both experiments concentrations of inoculum of 0; 4,500; 9,000 and 18,000 sporangia/plant were tested. Both experiments were set up in a split-plot design with sub-units in strips with

two replicates. Sub-units consisted of twelve seedlings. Papaya lines were allocated into the whole units. whereas the inoculum levels were allocated into the sub-units. Data on seedling mortality were taken and analysis of variance was performed according to the design used.

GENETICS OF ROOT ROT RESISTANCE IN PAPAYA

The seven papaya lines chosen from the 1974 planting and previous field information ranging from susceptible to resistant were grown and crossed, following a half diallel mating system (Jones, 1965). Many plants, however, were lost because of papaya mosaic virus, and it was possible to obtain only enough seed of a 5 x 5 half diallel which included lines 40, 'Waimanalo'-23, 45-T_{22}, 'Waimanalo'-24 and 'Higgins' as parental lines, and their 10 F_1 progenies for the genetic study.

Nursery stage

In order to estimate the components of genetic variance and determine heritability of resistance to root rot caused by P. palmivora in papaya, two experiments were set up.

The genetic materials included the 5 parental lines and their 10 F_1 hybrids. Two other cultivars, Line 8 and 'Kapoho' Solo, were included as controls. They were not, however, included in the half diallel analysis, but only in the analyses of variance corresponding to the field lay out employed.

One-month old seedlings were inoculated with 5,250 sporangia/plant in the greenhouse. Each experiment consisted of 17 treatments: Five lines and 10 F_1's of the diallel, and two check lines. There were five replicates for each experiment. One of the experiments had experimental

units of 24 plants each whereas the second had 50 plants in each unit.

One month after inoculation surviving seedlings were drenched with 24 ml of a suspension of Banrot 40W fungicide at 500 ppm to arrest the disease. Banrot 40W is a systemic fungicide composed of ETMT 15% and thiophanate methyl 25%.

ETMT is: 5-ethoxy-3-(trichloromethyl)-1,2,4,-thiadiazole. And thiophanate methyl is: dimethyl [(1,2,-phenylene) bis (imino-carbono thioyl)] bis [carbamate].

A third experiment which was not inoculated was set up outdoors in the nursery area on the Manoa campus. This experiment had experimental units of 24 plants and 5 replicates. Two of them were lost however, and data were obtained from only three replicates. The data from this experiment were used as a check to compare growth between healthy uninoculated plants and the inoculated plants of the other two experiments. A randomized complete block design was used for all three experiments.

Variables measured

In order to assess the degree of resistance of the papaya lines three variables were measured:

1. Percent mortality. Mortality counts were done weekly for four weeks. A plant was considered dead when only 3 fully expanded leaves remained.
2. Disease rating. Disease symptoms and vigor exhibited by the seedlings were rated 4 weeks after inoculation on a scale of 1 to 5 in which 1 = dead plant; 2 = weak plant with only chlorotic leaves and 75% defoliation; 3 = intermediate vigor

with 50% defoliation; 4 = vigorous plant with 25% defoliation and few chlorotic leaves; 5= vigorous plant with no apparent symptoms above ground.

3. Percent selectable plants. Selectable plants being those with vigor ratings of 4 and 5.

All three variables were measured taking into account the total number of plants in the experimental units. Percentages were transformed to angles, where angle = arc sin $\sqrt{\%}$ to stabilize variances, according to Snedecor and Cochran (1967). Transformed percent mortality and disease ratings were analyzed by the analysis of variance of the lay out and the genetic analysis of the half diallel. Transformed percent selectable plants was not analyzed genetically.

Other variables were measured to evaluate the extent to which growth was reduced by root rot. Characteristics measured were number of nodes per plant, number of leaves per plant (excluding cotyledonary leaves), plant height in centimeters from the cotyledonary node to the stem apex, and stem diameter in millimeters at the cotyledonary node. Measurements were taken when the papaya plants were one month old, prior to inoculation, and again one month after inoculation. Because of the large number of plants in the experiments and the need to record the data in the shortest possible time to keep variation to a minimum, measurements were made on 20 plants chosen at random from each experimental unit with 50 plants, and 10 plants in experiments with 24 plants.

Analyses of variance were made on the data from the following variables:

1. Increase in number of nodes per plant. This was calculated by

subtracting number of nodes per plant of 1-month old plants from the number of nodes per plant of 2-month old plants.

2. Number of leaves lost per plant. This variable was calculated as follows: $Ll_{1-2} = El_2 - Al_2$ and $El_2 = Nl_1 + \Delta n_{1-2}$

where: Ll_{1-2} = number of leaves lost in 30 days between the end of 1st and the end of 2nd month of growth.

El_2 = expected number of leaves at the end of the 2nd month of growth.

Al_2 = actual number of leaves present at the end of the 2nd month of growth.

Nl_1 = number of leaves present at the end of the 1st month of growth.

Δn_{1-2} = increase in number of nodes in 30 days between the end of the 1st and the end of the 2nd month of growth.

3. Percent defoliation. This was calculated as:

$$\% D = \frac{(Ll_{1-2})\ 100}{El_2}$$ where % D is percent defoliation.

4. Plant height. At 1 and 2 months after germination.

5. Stem diameter. At 1 and 2 months after germination.

6. Increase in plant height. Subtracting height of 1-month old plants from height of 2-month old plants.

7. Increase in stem diameter. Subtracting stem diameter of 1-month old plants from stem diameter of 2-month old plants.

It should be pointed out that at the end of the second month of growth the variables were recorded only for plants surviving the

inoculation, except for number of leaves and percent defoliation in which dead plants were also included since by a previous definition, they were considered dead when only three leaves remained attached to the plant.

To summarize: analyses of variance were carried out for percent mortality, disease rating, percent selectable plants, increase in number of nodes per plant, number of leaves lost per plant, percent defoliation, plant height, stem diameter, increase in plant height and increase in stem diameter. Tests of significance were done using the K-ratio t test between lines under similar disease conditions. Correlation coefficients between percent mortality and disease ratings; between percent mortality and percent selectable plants; and between percent selectable plants and disease ratings were calculated.

A correlation coefficient between percent mortality in inoculated 1-month old seedlings of seven papaya lines and percent mortality obtained in four continuous field plantings of the same lines was calculated to confirm reliability of the inoculation method used.

Comparison of each papaya line and F_1 hybrid under inoculated and uninoculated conditions was done by using the method of comparison of groups of unequal sizes. Variables compared were those reflecting plant growth, and defoliation. Variances within each line were used for the test of significance rather than the pooled variance for entire experiments. Cochran's t' was used rather than Student's t in the few cases of heterogeneity of variances (Snedecor and Cochran, 1967).

Genetic analyses

Data on percent mortality transformed to angles, and disease

ratings for the parental lines and their F_1 hybrids obtained following a half diallel crossing system were analyzed in two different ways:

1. Analysis followed basically Hayman's method (1954a, 1954b, and 1960), with the modifications by Jones (1965):

   The phenotypic variation was partitioned into the following components of genetic and environmental variation:

   D = variation due to additive effects of the genes.

   H = variation due to dominance effects of the genes.

   $H_2 = H_1 (1 - (u-v)^2)$ where: u = proportion of positive genes in the parents.

   and v = proportion of negative genes in the parents.

   h = dominance effect over all loci in heterozygous condition in all the crosses.

   F = mean over parental arrays of the covariance of additive and dominance effects.

   E = environmental component of variation.

Using the above partitioned components, heritability was calculated in both narrow and broad senses using the formulas of Mather and Jinks (1971):

$$\text{Narrow Heritability} = \frac{\frac{1}{2}D + \frac{1}{2}H_1 - \frac{1}{2}H_2 - \frac{1}{2}F}{\frac{1}{2}D + \frac{1}{2}H_1 - \frac{1}{2}H_2 - \frac{1}{2}F + E} \quad \text{and}$$

$$\text{Broad Heritability} = \frac{\frac{1}{2}D + \frac{1}{2}H_1 - \frac{1}{2}H_2 - \frac{1}{2}F}{\frac{1}{2}D + \frac{1}{2}H_1 - \frac{1}{2}H_2 - \frac{1}{2}F + E}$$

It was also possible to assess the mean degree of dominance with the formula $\sqrt{H_1/D}$; the proportion of genes with positive and negative effects in the parents by the formula, $H_2/4H_1$; and the proportion of

dominant and recessive genes in the parents by the formula, $\frac{\sqrt{4DH_1 + F}}{\sqrt{4DH_1 - F}}$

The graphic V_r, W_r (V_r = variance of the r^{th} array; W_r = covariance between parents and their offspring in the r^{th} array) analysis was also carried out so that the relative positions of parents with more dominant genes and the ones with more recessive genes could be identified.

2. Method 2, Model II of Griffing's (1956a) was used to analyze the data.

   Treatment sum of squares was partitioned into general combining ability and specific combining ability so that genotypic variance and additive variance, as well as phenotypic and environmental variance could be calculated to estimate both narrow and broad sense heritability.

   Symbols and formulas used:

   σ^2_g = general combining ability

   σ^2_s = specific combining ability

   σ^2_A = additive genetic variance

   σ^2_G = genotypic or total genetic variance

   σ^2_P = phenotypic variance

   σ^2_e = environmental variance

Additive genetic variance is estimated by: $\sigma^2_A = 2\,\sigma^2_g$

Genotypic variance is estimated by: $\sigma^2_G = \dfrac{2\,\sigma^2_g}{2\,\sigma^2_g + \sigma^2_s}$

Phenotypic variance is estimated by: $\sigma_P^2 = 2\ \sigma_g^2 + \sigma_s^2 + \sigma_e^2$

Narrow heritability = $\frac{\sigma_A^2}{\sigma_P^2}$ and Broad Heritability = $\frac{\sigma_G^2}{\sigma_P^2}$

Follow up after transplanting in the field

An experiment was designed to evaluate the performance of the surviving seedlings resulting from inoculations and to compare them with uninoculated seedlings.

Seedlings were transplanted at Waimanalo Research Station into field Q-1 which has been planted to papayas for 8 successive plantings one week after the inoculated seedlings were drenched with fungicide. P. palmivora had been previously confirmed in field Q-1 (Nakasone and Aragaki, 1973). Two plants per hole were planted in rows spaced 3.0 m apart and 0.9 m in the row. The plants were rogued 3 months after transplanting to leave 1 plant per hole. The alternate plants were removed one month later to leave a spacing between plants of 1.8 m which is considered an adequate spacing for papaya breeding experiments. Fertilizer was applied every two months using approximately 500 grams per plant of 10-10-10 fertilizer. The field was furrow irrigated every two weeks except during rainy periods.

A split-plot design was utilized in the field planting. Each unit consisted of 24 plants (2 plants/hole) of a parental line or hybrid. Every unit was divided into 2 sub-units consisting of a set of survivors from the inoculation done when the seedlings were one month old, and another set of healthy plants which had not been inoculated. Sub-units had each 12 plants at the beginning of the experiment. Plants were

rogued to 1 plant per hole 3 months after transplanting, followed by a 2nd roguing after 1 additional month. This left 6 plants per unit and 3 plants in each sub-unit.

Due to losses in the nursery there were only three replicates. Replications were located in the field according to historical information on mortality ratings on the previous planting. In other words they were located in areas where the disease had occurred before.

Mortality counts were taken monthly during the first 3 months after transplanting. At the end of the fourth month after transplanting disease symptoms and vigor of plants were rated on a scale from 1 to 5. Plant height and stem diameter in cm and mm were recorded, one, 3, and 4 months after transplanting, and analysis of variance was calculated for each date.

A similar experiment except for reduction in size of the experimental units to one half, was planted on the same date in another field in which papayas had not been planted for about 15 years and was assumed to be free of P. palmivora. The objective was to estimate the degree of effectiveness of field infection by comparing the two sets of uninoculated papaya lines under both infested and noninfested fields. Comparison between the two sets of survivors under the same conditions specified above would have provided information on the carry-over effect of inoculation done in the greenhouse prior to transplanting in the field.

Unfortunately data from this experiment were incomplete because of vandalism. Thirteen sub-units were completely lost and in four sub-units more than 50% of the plants were chopped off. This incident occurred one and a half months after transplanting. Sixty six percent of the

remaining plants in this field were attacked by papaya mosaic virus. So this planting had to be abandoned.

RESULTS AND DISCUSSION

ASSESSMENT OF ROOT ROT RESISTANCE IN PAPAYA

Field study

From the 27 papaya lines planted in the replant O-1 at Waimanalo during 1974, data on percent mortality and disease ratings for 9 of these lines are presented in Table 2. Mean percent mortality for three previous plantings (Nakasone and Aragaki, 1973) and that of the 1974 planting are also included for most of the lines.

Two groups could be formed from the 1974 percent mortality and disease ratings: 'Higgins', 25, 'Kapoho' Solo and 'Waimanalo'-24 as susceptibles; and Lines 45-T_{22}, 41, 'Waimanalo'-23 and 40 as resistant. However, taking into account the means of three previous plantings and those of 1974 a more precise classification of the 9 papaya lines with respect to their response to root rot under replant field conditions may be done and they would be regrouped as follows: susceptible lines: 'Higgins' and 25; moderately resistant: 'Kapoho' Solo, 45-T_{22}, and 'Waimanalo'-24; and resistant lines: 8, 41, 40 and 'Waimanalo'-23.

Data in Table 2 show that the percent mortality in 1974 was much higher for every line than the mean for four consecutive plantings. Nakasone and Aragaki (1973) have discussed variation in mortality from one planting season to the next as a difficulty in breeding for resistance to Phytophthora root rot. The 1974 results appear to confirm this when compared to the previous plantings.

In spite of the generally high mortality in the 1974 planting, Line 25, 'Higgins', and 'Kapoho' Solo showed the highest mortality whereas 'Waimanalo'-23, showed low mortality. This agrees with Nakasone

TABLE 2. -- Percent mortality and disease ratings on papaya lines in 1974, and mean percent mortality in 1971, 1972, 1973 and 1974

Lines	% mortality, 1974 Transf. arc sin $\sqrt{\%}$	% mortality, 1974 Retransf. %	Disease rating[x] 1974	Mean of 4[y] plantings % mortality
Higgins	74.3	94.7	1.6	58.6
25	55.2	67.4	2.5	58.7
Kapoho Solo	50.8	60.1	2.5	41.1
Waimanalo-24	50.0	58.6	2.5	----
Line 8	44.3	48.8	3.1	18.2
45-T_{22}	40.6	42.3	3.2	----
41	31.7	27.6	3.7	14.4
Waimanalo-23	28.8	23.2	3.7	9.2
40	27.3	21.1	3.6	19.7
K-ratio t (0.05)	32.0		1.7	

[x]Rating scale measured from 1 = dead plant, to 5 = healthy vigorous plant

[y]Percent mortality for 1971, 1972 and 1973 taken from Nakasone and Aragaki (1973)

and Aragaki's report (1973).

Inasmuch as the 9 papaya lines reported in Table 2 possess desirable horticultural and fruit characteristics with the added feature of having different degrees of resistance to Phytophthora root rot, they were selected for further study in artificial inoculations to determine more precisely their resistance response and the genetics of resistance.

Greenhouse studies

A. Laboratory-greenhouse method of assay.

1. Determining optimum concentration of inoculum and time of exposure to cause a specific mortality percentage in a susceptible papaya line.

Analysis of variance of the percent mortality obtained by testing concentrations of inoculum and time of exposure of seedlings to the inoculum indicate that in partitioning the sum of squares for treatments, all comparisons except for the linear regression for concentration of inoculum tested during 4 hours were highly significant at the 1% level. This showed significance only at the 5% level of probability (Table 3). The high sum of squares from the comparison of controls vs. inoculated treatments was due to lack of dead plants in the control group and high mortality in the inoculated treatments. Two of the control treatments were comprised of seedlings immersed for 2 and 4 hours in deionized water and subjected to the hypochlorite rinse for 10 minutes. This result confirms Aragaki's (1975) finding that hypochlorite does not produce toxic effects in surface disinfesting papaya seedlings.

TABLE 3. -- Degrees of freedom and mean squares for percent mortality, and regression equations in an experiment testing time of exposure and concentration of inoculum in papaya cv Higgins

Source of variation	df	ms
Replicates	3	516.5 ns.
Treatments	(8)	6,764.6 **
Controls vs. inoc. treat.	1	46,240.9 **
Inoc. 2-hr vs. 4-hr.	1	3,152.7 **
Inoc. 2-hr linear regression	1	2,514.7 **
Inoc. 4-hr linear regression	1	1,490.2 *
Dev. from regression	4	179.5 ns.
Error	24	303.7

*Significance at 5% level.
**Significance at 1% level.
ns. No significance.

Regression equations for 2-hour and 4-hour inoculations:

	Complete model	Reduced model
2-hour:	$\hat{Y} = 37.48 + 11.61\ X$	$\hat{Y} = 24.3\ X$
4-hour:	$\hat{Y} = 66.64 + 8.94\ X$	$\hat{Y} = 31.2\ X$

$\hat{Y}$ = % mortality; X = sporangia/ml; X = 1 = 250 sp/ml.

Significance of the comparison of inoculated treatments for 2 hours vs. 4 hours shows that with increasing time of exposure, a decreasing concentration of inoculum is required to cause a specific mortality rate in papaya seedlings. This confirms the finding of Aragaki (1975).

The comparisons which are most relevant to the main objective of this experiment are the significant mean squares for the linear regressions of percent mortality on concentration of inoculum for both 2-hour and 4-hour inoculated treatments. The 2-hour inoculation time is more practical than the 4-hour inoculation due to saving of time permitting inoculation of larger number of seedlings, although higher concentrations of inoculum were required to achieve the same levels of mortality (Fig. 3). The fitted regression lines passing through the point of origin are shown in Fig. 3. The complete models and reduced models are shown in Table 3.

The 2-hour inoculation method was selected for further experiments. The reduced model was employed to calculate a concentration of inoculum which would kill 70% of the seedlings of cv. Higgins. The concentration obtained was 720 sporangia/ml. The choice of 70% mortality was arbitrary. It was, however, hypothesized that if such a concentration were to be used in testing more resistant papaya lines, those lines would have lower mortality than that of 'Higgins', and hopefully, not less than 30% so that analysis of variance could be performed without problems of heterogeneous variances, and further avoiding the need of using transformation of the data.

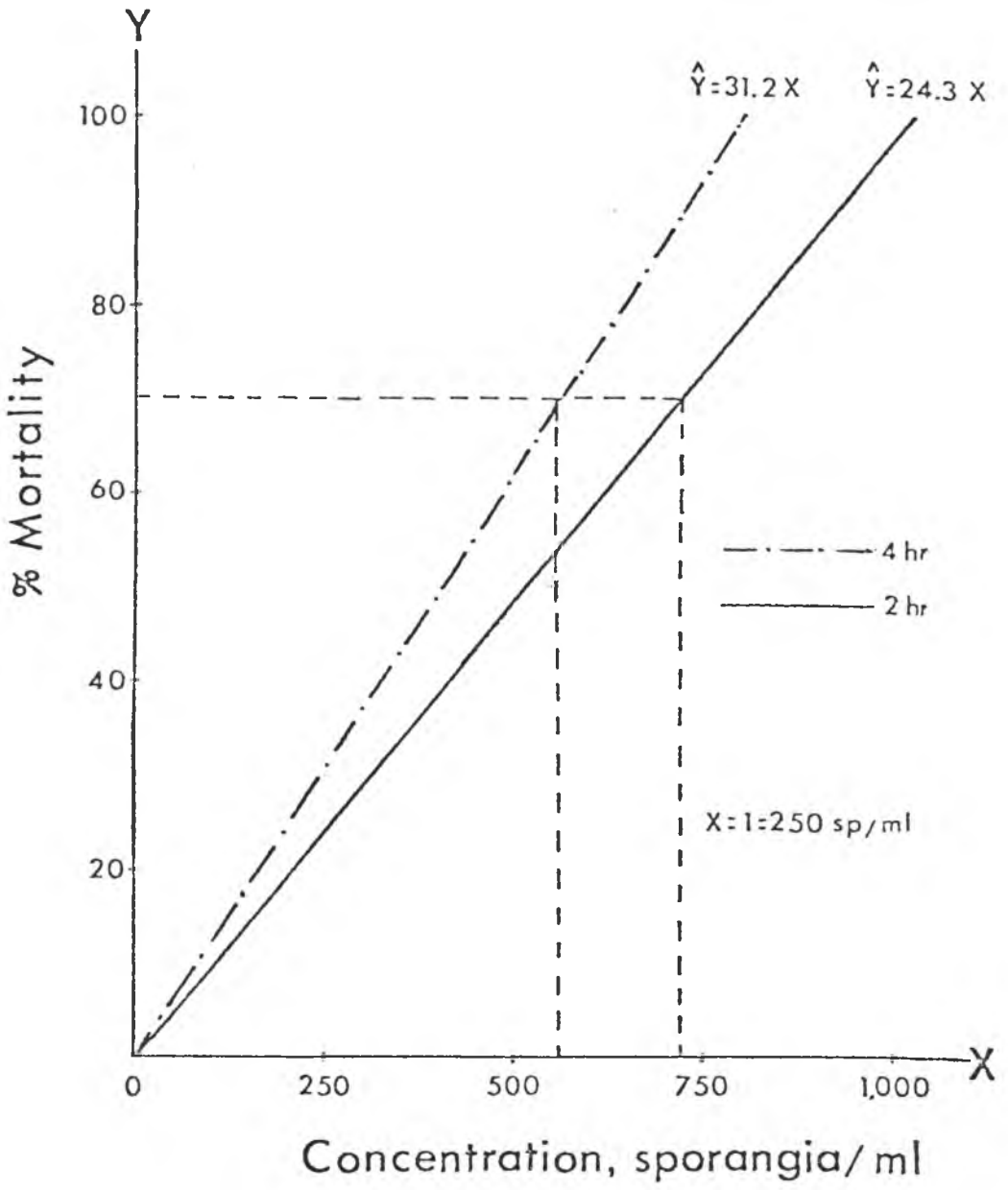


Fig. 3 Regression of percent mortality of papaya seedlings of cv. Higgins on concentration of P. palmivora sporangia

2. Comparison of 7 papaya lines ('Solo' type) with cv. Higgins for their response to inoculation by the laboratory-greenhouse methods.

All paired comparisons were planned between the seven lines and cv. Higgins which became the standard cultivar for susceptibility in most experiments. The data in Table 4 shows only Line 40 to have significantly higher mortality than 'Higgins', as shown by the K-ratio t test.

'Sunrise' Solo, which was used by Aragaki (1975) in developing his method of assay, showed 68.7% mortality, which compares well with the 60% mortality in a 2-hour inoculation reported by Aragaki (1975) working with the same Phytophthora isolate used in this study, although he did not indicate the concentration used.

The correlation between percent mortality in the field (mean of 4 plantings, Table 2) and percent mortality in this experiment was $r = -0.3024$ which was not significantly different from zero, meaning that there was no correlation between laboratory and field results.

Line 40, which had been classified as showing resistance in the field, showed the highest mortality in this laboratory test. Further testing of Line 40 and 'Higgins' was therefore indicated.

3. Further comparison of response to laboratory-greenhouse inoculation at different concentrations of inoculum between Line 40 and 'Higgins'.

Fitted regression lines of percent mortality on

TABLE 4. -- Percent mortality in 8 papaya lines inoculated with 720 sp/ml of P. palmivora using laboratory-greenhouse inoculation method

Lines	% mortality $\bar{X} \pm S_{\bar{x}}$		No. of reps.	K-ratio t (0.05)
40	77.3	6.2	6	lines w/6 reps.
Waimanalo-23	70.8	7.1	6	23.4%
Sunrise Solo	68.7	8.6	4	
25	62.5	7.2	4	lines w/7 reps.
Waimanalo-24	57.0	10.4	6	22.5%
45-T_{22}	47.6	3.5	7	
41	42.8	9.0	7	lines w/4 reps.
Higgins	50.0	5.9	5	25.8%

Correlations between percent mortality in the field for 4 plantings (Table 2) and percent mortality in this experiment: $r = -0.3024$ ns

concentration of inoculum for both Line 40 and 'Higgins' are shown in Fig. 4. The reduced model is shown in the graph since the elevations did not differ significantly from zero. There was no significant difference between the regression lines for the two papaya lines (Table 35), suggesting that Line 40 had about the same degree of susceptibility as 'Higgins' to P. palmivora.

From the results of these experiments there appear to be three possible explanations to the lack of significant differences between the papaya lines tested: (1) the classification of the lines on the basis of field results may have been inadequate, which would reflect a close genetic relationship among lines derived from common sources of the 'Solo' papaya type (Nakasone, 1975); (2) the method of inoculation used may have been ineffective; or (3) there is no resistance at the age of the seedlings tested. Before changing methods of inoculation and testing different ages of seedlings, an experiment was carried out using foreign accessions from a different gene pool.

4. Comparison of papaya seedlings of foreign accessions against 'Higgins' by their response to laboratory-greenhouse inoculation.

Mean percent mortality obtained from 10 papaya lines from different geographical locations showed five lines with similar mortality to 'Higgins' and 4 appeared more susceptible than 'Higgins' (Table 5). The number of foreign papaya lines tested was not large enough and even though none showed more resistance

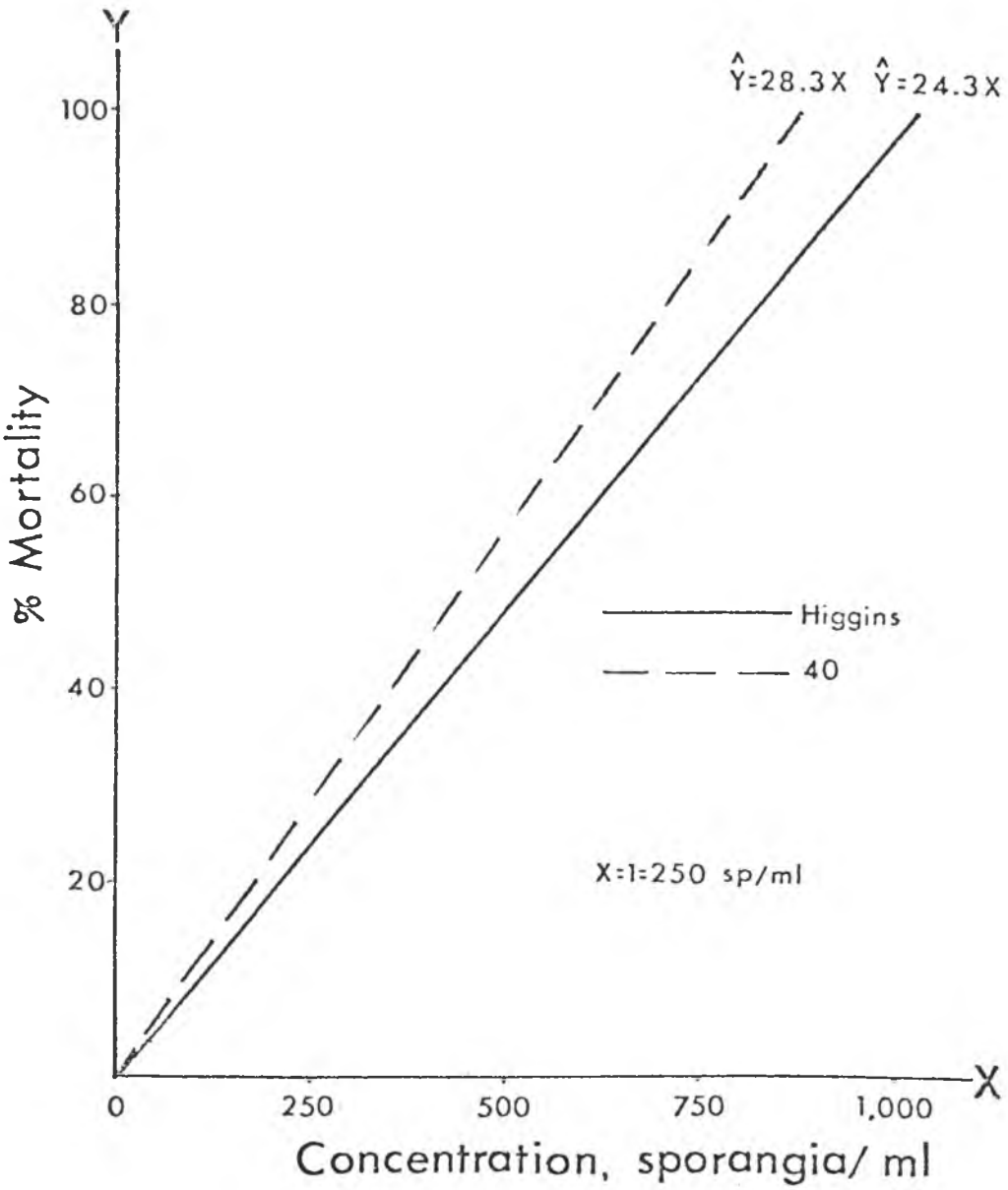


Fig. 4 Regression of percent mortality on concentration of P. palmivora sporangia in two papaya lines

TABLE 5. -- Mean percent mortality obtained in 10 papaya accessions inoculated with 720 sp/ml of P. palmivora using laboratory-greenhouse method

Papaya accessions	% mortality	No. of replicates
L-3-6	97 *	4
L-18-16	96 *	4
HAES 8018	96 *	4
Line 83 (OP)	94 *	3
L-15-M	92	3
Line 122 (OP)	88	4
L-1-17	83	4
72-8(71-4) T OP	78	4
L-15-14	68	4
Higgins	71	4

*Accessions with mortality significantly higher than 'Higgins' as tested with K-ratio t test (0.05).

than 'Higgins' so it is not possible, to arbitrarily conclude that there is no resistance to P. palmivora in the species C. papaya L. It is possible however that some foreign papaya lines showed greater susceptibility than 'Higgins' because the isolate of the fungus used was locally obtained from papaya roots in Hawaii (Aragaki, 1975). The Hawaiian isolate may be highly virulent to papaya lines native to some other geographical areas. Blaha (1974) pointed out that in searching for resistance to P. palmivora in cacao, local isolates should be used, because they have probably evolved in the environment where the host has been cultivated for many years.

B. Testing a different method of inoculation.

In order to test the alternative hypothesis that the failure to detect differences in susceptibility between papaya lines was due to age at which seedlings were inoculated, a different method of inoculation was developed. A new series of experiments was initiated.

1. Comparing 4 levels of inoculum on seedlings of 'Higgins' at 4 different ages using the greenhouse inoculation method.

Data in Fig. 5 show that the slopes of the regression lines are greater for younger seedlings than for older ones. This indicates that higher concentrations of inoculum were necessary to induce a given percentage of mortality as seedlings become older. Regardless of the age at which seedlings were tested, symptoms on the stem portion usually appeared the third day after inoculation. There were, however, differences in the type of symptoms, depending on the age of

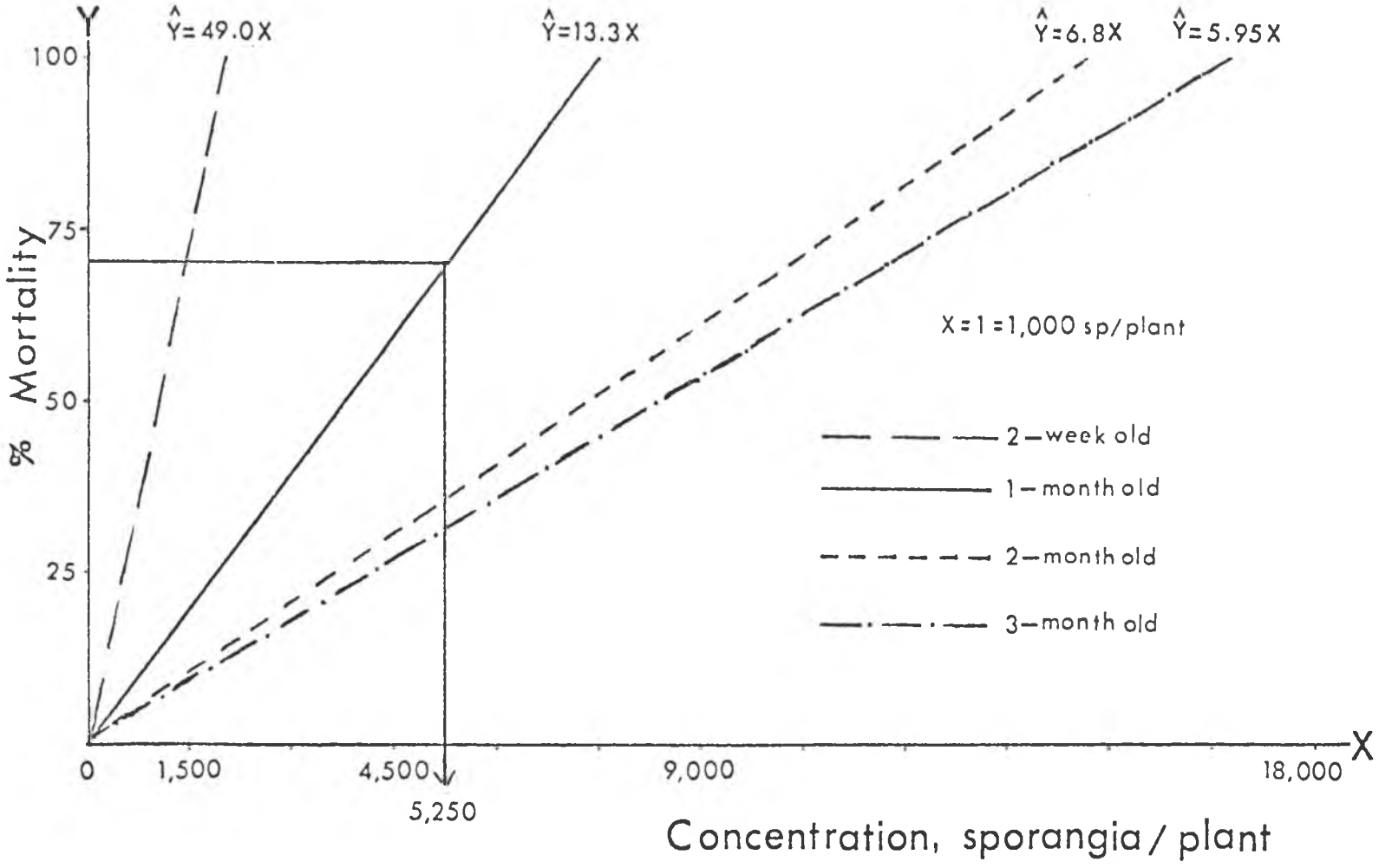


Fig. 5 Regression of percent mortality on concentration of P. palmivora sporangia of cv. Higgins at 4 different ages

the seedlings. Symptoms in 1- and 2-week old seedlings resembled those described by Chee (1974) as seedling blight with sudden and conspicuous withering, termination of growth activity, and necrosis of plant parts without rotting. This occurred on leaves but not on the roots which actually rotted. Symptoms in 1-week and 2-week old seedlings are shown in Figs. 6, 7, 8 and 9. Symptoms on leaves, stems and petioles of seedlings 1-month of age and older closely resembled those occurring under field conditions described by Parris (1942) and Trujillo and Hine (1964), except at very high levels of inoculum when seedlings wilted rather suddenly. The death of 1 and 2-month old seedlings did not occur until after the fifth or sixth day after inoculation at all levels of inoculum tested. In 3-month old seedlings death occurred at the 7th day after inoculation at the highest level of inoculum, and at the 12th to 14th day with lower levels of inoculum. Symptoms on 1-month old seedlings of 'Higgins' are shown in Figs. 10 and 11; those of 2-month old seedlings in Figs. 12 and 13; and those of 3-month old seedlings in Figs. 14 and 15.

In this study motile zoospores of isolate P170 of P. palmivora were the infective propagules used, although the concentrations of inoculum are reported as sporangia/plant.

Relationship between higher susceptibility of papaya seedlings to P. palmivora at younger growth stages has been shown previously by KO (1971) in Hawaii and Ramirez and Mitchell (1975) in Florida. In both instances they reported using unspecified 'Solo' type cultivars. Inoculations were made with

PLATE I

Seedling blight symptoms on one and 2-week old papaya seedlings, and root rot symptoms on one, 2 and 3-month old seedlings of 'Higgins'

Figure 6. One-week old seedlings, one week after inoculation

Figure 7. One-week old seedlings, one month after inoculation

Figure 8. 2-week old seedlings, one week after inoculation

Figure 9. 2-week old seedlings, one month after inoculation

Figure 10. 1-month old seedlings, one week after inoculation

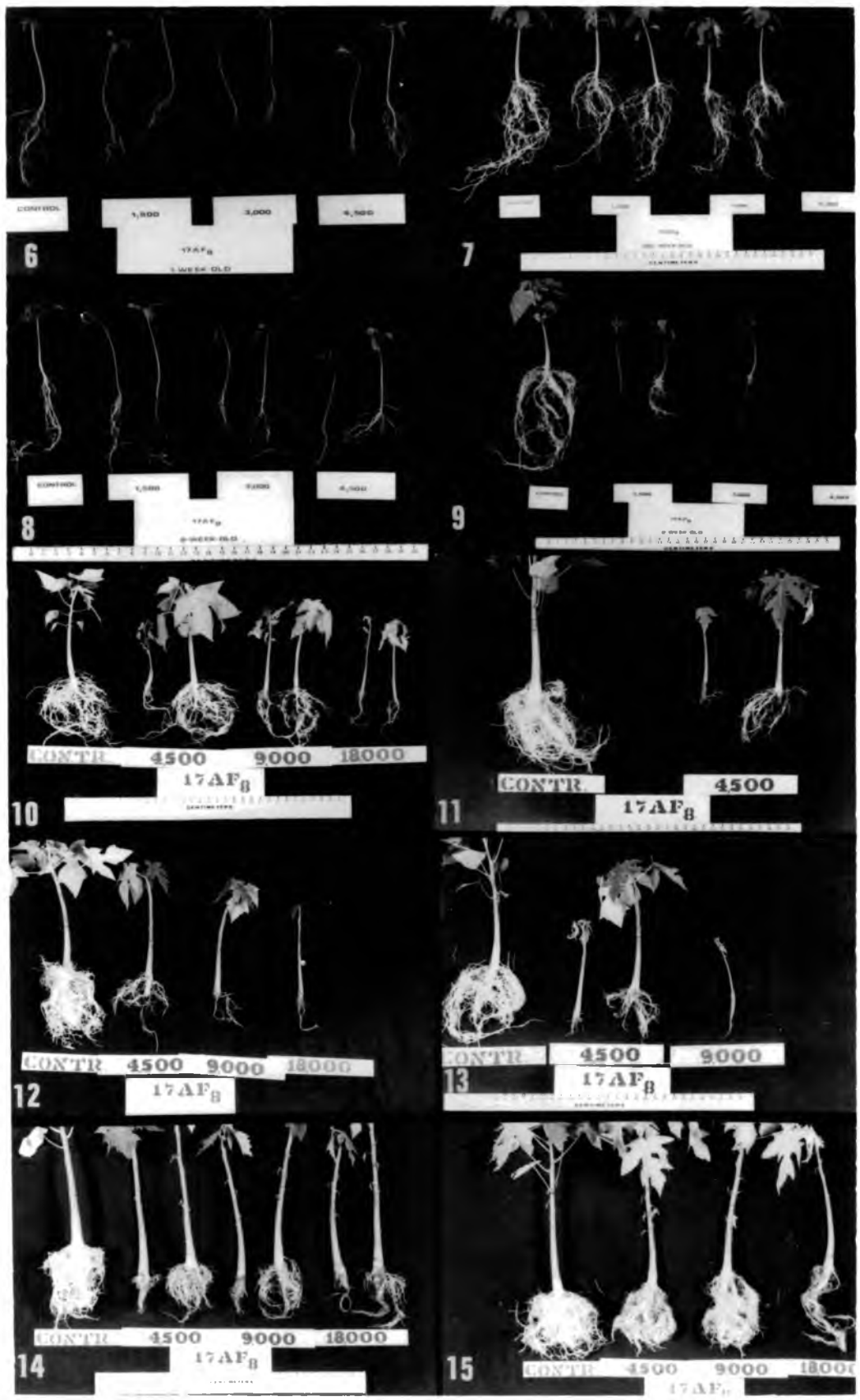
Figure 11. 1-month old seedlings, one month after inoculation

Figure 12. 2-month old seedlings, one week after inoculation

Figure 13. 2-month old seedlings, one month after inoculation

Figure 14. 3-month old seedlings, one week after inoculation

Figure 15. 3-month old seedlings, one month after inoculation


6
7
8
9
CONTR 4500 9000 18000
17AF8
10
CONTR 4500
17AF8
11
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17AF8
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CONTR 4500 9000
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CONTR 4500 9000 18000
15

zoopores and other types of propagules of the pathogen. In Florida the isolate used was different from that used in the present study. In the present study vermiculite was the media used to grow the seedlings. In studies reported by Ko (1971) and Ramirez and Mitchell (1975) autoclaved soil was used. In any case the results of those workers were confirmed. In comparing the regression lines for different ages of seedlings shown in Table 36, mortality was significantly different at all levels tested between 2-week, 1-month, and 2-month old seedlings. There was no significant difference between 2-month and 3-month old seedlings at all levels of inoculum tested. This indicates that at these levels of inoculum, 2-month old seedlings have fully developed their mechanism of resistance. Ko (1971) indicated that resistance continues to increase until the seedlings reach an age of three months. Data in Fig. 5 suggest that the regression equation for 1-month old seedlings may be used to predict the concentration (5,250 sporangia/ plant) required to cause 70% mortality in 'Higgins'. The predicted concentration of inoculum was intended for use in the genetic study of resistance. It is pointed out here that this study was carried out with one of the most susceptible lines of the 'Solo' type and that the resistance is relative in this context to the developmental stage of the plant. This increase in resistance as seedlings become older may be called developmental resistance. It is possible that is plant size rather than age which is related to susceptibility or resistance to the disease. It is easier, however, to make

reference to age since size is strongly affected by other environmental factors. It is clear, however, that a character should be looked for that expresses better than age such relationship between seedling size and resistance.

The fact that results comparable with those of Ko (1971) and Ramirez and Mitchell (1975) using autoclaved soil were obtained demonstrates that vermiculite is a satisfactory medium to grow papaya seedlings for pot inoculations with zoospore suspensions. Turner and Asomaning (1962) used washed sand and sterilized soil as media to grow cacao seedlings to inoculate with P. palmivora. Zentmyer et al. (1968) prefer the use of Hoagland's nutrient solution in growing cacao plants for inoculation. The experiments reported here indicate that vermiculite which needs no previous sterilization is a satisfactory media to use in inoculation tests.

2. Determining the minimum concentration of inoculum at which a significant response in mortality is obtained in 1-week old seedlings of 'Higgins'.

Data presented in Table 6 show an increase in mortality in 1-week old seedlings of 'Higgins' with increasing concentrations of inoculum (Fig. 16). Williams' $\bar{t}$ test (1971) indicates however, that there is evidence for a significant level of mortality only at 200 sporangia/plant when compared to the control which had no mortality. Ramirez and Mitchell (1975) reported that mortality of 45-day old papaya seedlings planted in soil, was first observed with levels as low as 10^4 zoospores per plant. Considering that the number of zoospores released

TABLE 6. -- Mean percent mortality in 1-week old papaya seedlings of 'Higgins' inoculated with increasing concentrations of P. palmivora. Sequential Williams' $\bar{t}$ test

Concentration of inoculum sp/plant	Percent mortality	Transformed arc sin $\sqrt{\%}$	Calculated[x] $\bar{t}_k$	Tabulated 0.05	$\bar{t}_k$ 0.01
0	0	0.81			
8	25.0	22.91	$\bar{t}_1 = 1.30$	2.02	3.36
40	37.5	35.72	$\bar{t}_2 = 2.05$	2.14	3.50
200	50.0	45.00	$\bar{t}_3 = 2.59$*	2.19	3.55
1,000	70.8	60.99	$\bar{t}_4 = 3.53$*	2.21	3.57
5,000	100.0	81.87	$\bar{t}_5 = 4.75$**	2.22	3.59

[x]Comparison between each concentration tested vs. the zero control

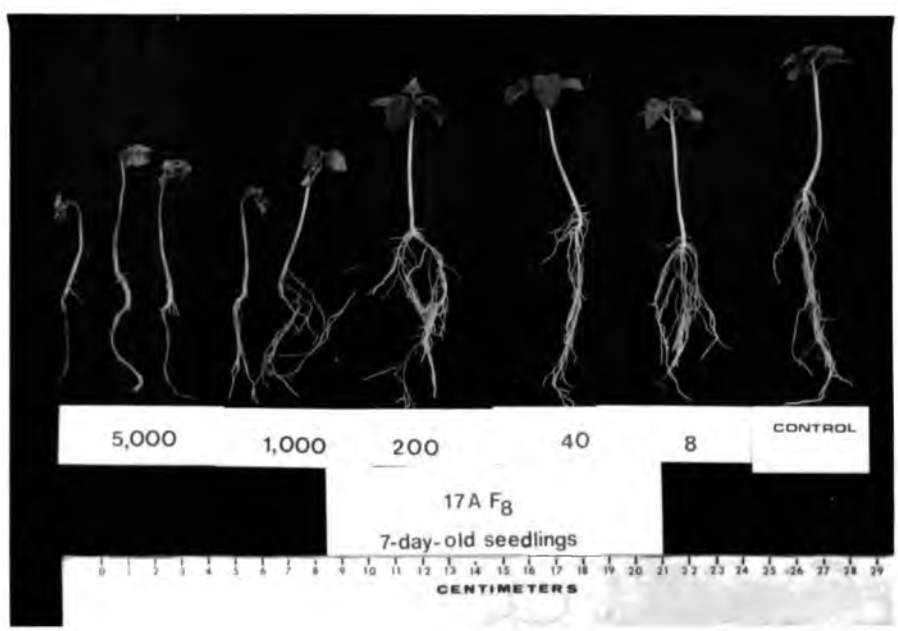


Fig. 16. Seedling blight symptoms shown by one-week old seedlings inoculated with low levels of inoculum

per sporangia reported to be 16 by Ko and Chan (1974) is a reasonable estimate for P. palmivora it would mean that 3,200 zoospores is the lowest concentration needed to cause a significant mortality in 'Higgins' seedlings. This agrees to some extent with the results of Ramirez and Mitchell (1975) taking into consideration that the age of the seedlings tested in this experiment was only 1 week compared to 45 days. This stresses the relationship of seedling age-susceptibility previously demonstrated in this report and those of Ko (1971) and Ramirez and Mitchell (1975).

The presence of dead plants at levels of inoculum as low as 8 sporangia per plant indicates that infection took place. This was demonstrated by reisolation of the pathogen by plating roots from plants 1 week after inoculation. It has been demonstrated in fruits and leaves of papaya that even a single zoospore can cause infection (Doo, 1964). In the present study, however, no attempts were made to assess percent infection. Williams' (1971) $\bar{t}$ test to compare differences between treatment means in experimenting with increasing concentrations of inoculum using a zero control is useful when the objective is to determine the lowest concentration of inoculum to obtain a significant response.

3. Testing three inoculum levels on 1 and 2-week old seedlings of 'Higgins', 'Kapoho' Solo and 'Waimanalo'-23.

Previous experiments inoculating seedlings of 'Higgins' demonstrated that root rot resistance increases as seedlings become older. To determine whether or not the mechanism of

resistance is on in 1 and 2-week old seedlings, three cultivars were chosen on basis of their field resistance. 'Waimanalo'-23 as resistant, 'Kapoho' Solo as moderately resistant and 'Higgins' as the susceptible check.

The linear regression equations of percent mortality on concentrations of inoculum in the three cultivars inoculated at 1 week after germination are shown in Fig. 17. Regression for the three cvs. were not significantly different as shown in Table 37. Similar result was found for seedlings inoculated 2 weeks after germination and this is shown in Fig. 18 and Table 38. Comparisons made for the same cultivar between the two ages tested did not show significant differences either, as indicated in Table 39. Figures 19 to 24 show 1-week old seedlings and Figures 25 to 30 show 2-week old seedlings of the three cultivars.

This experiment demonstrated that resistance does not start acting until after the second week after seedlings germinate. This also explains why the inoculation method devised by Aragaki (1975) failed to detect differences between resistant and susceptible papaya lines.

4. Testing three concentrations of inoculum on 1 and 2-month old seedlings of several papaya lines.

Results of previous experiments with the new method of inoculation indicated a need to determine whether other papaya lines would also show developmental resistance with increased age as shown by 'Higgins'. It would be desirable also to show whether or not there were lines having higher resistance than

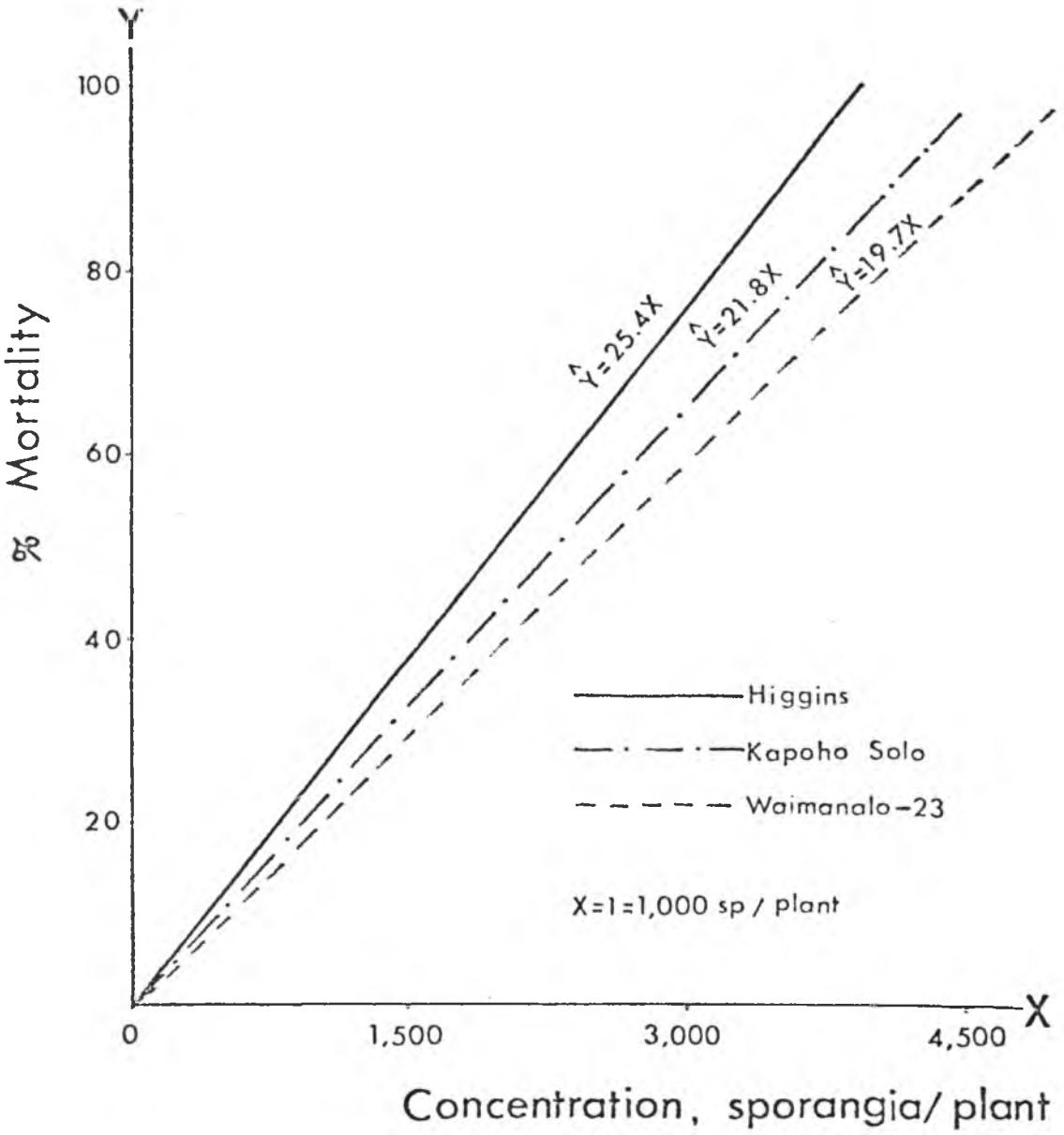


Fig. 17 Regression of percent mortality on concentration of P. palmivora sporangia in 1-week old seedlings of three papaya lines

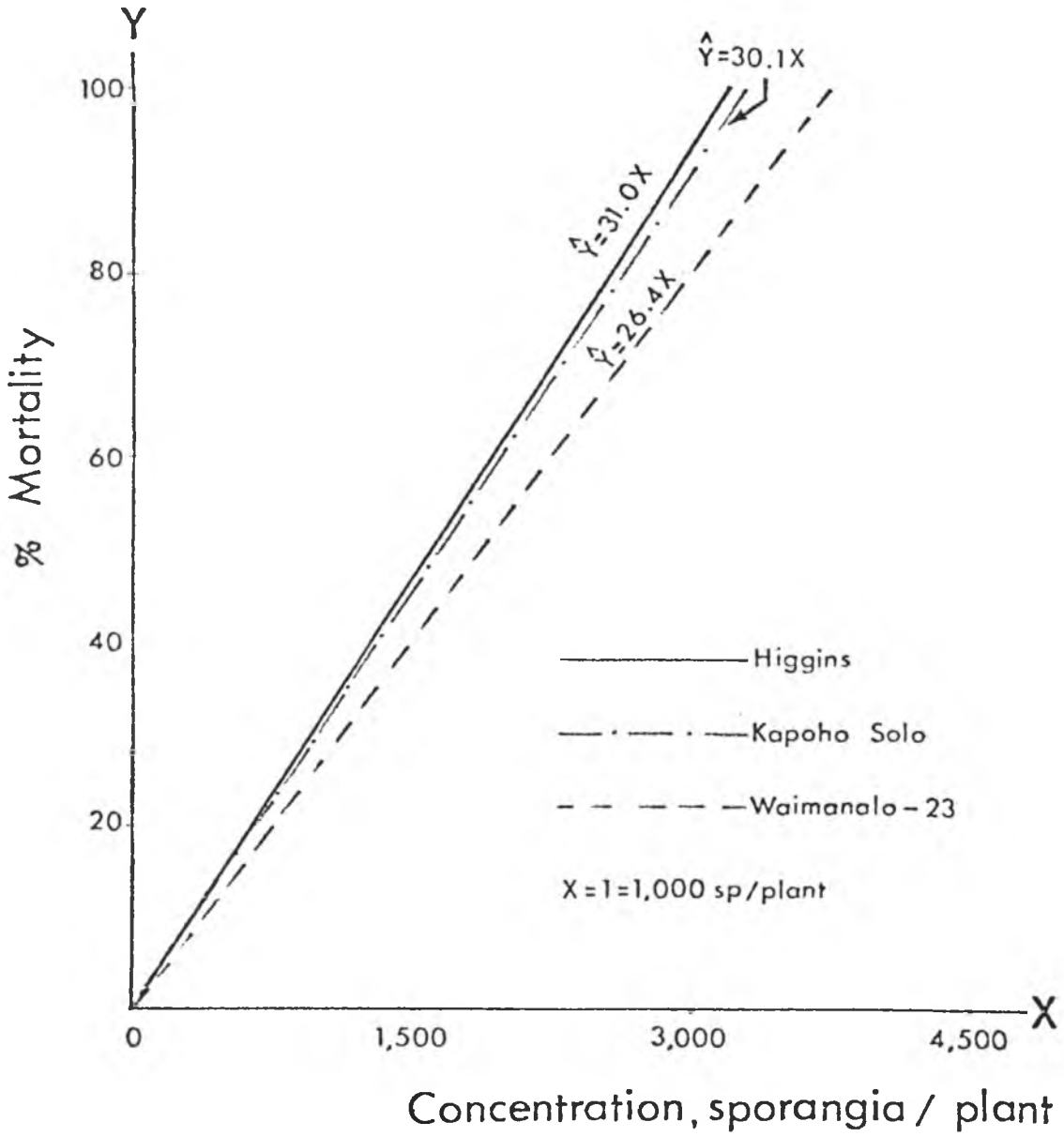


Fig. 18 Regression of percent mortality on concentration of P. palmivora sporangia in 2-week old seedlings of three papaya lines

PLATE II

Experiment testing three inoculum levels on one and 2-week old seedlings of three cultivars

Figure 19. One-week old seedlings of 'Higgins', one week after inoculation

Figure 20. One-week old seedlings of 'Higgins', one month after inoculation

Figure 21. One-week old seedlings of 'Kapoho' Solo, one week after inoculation

Figure 22. One-week old seedlings of 'Kapoho' Solo, one month after inoculation

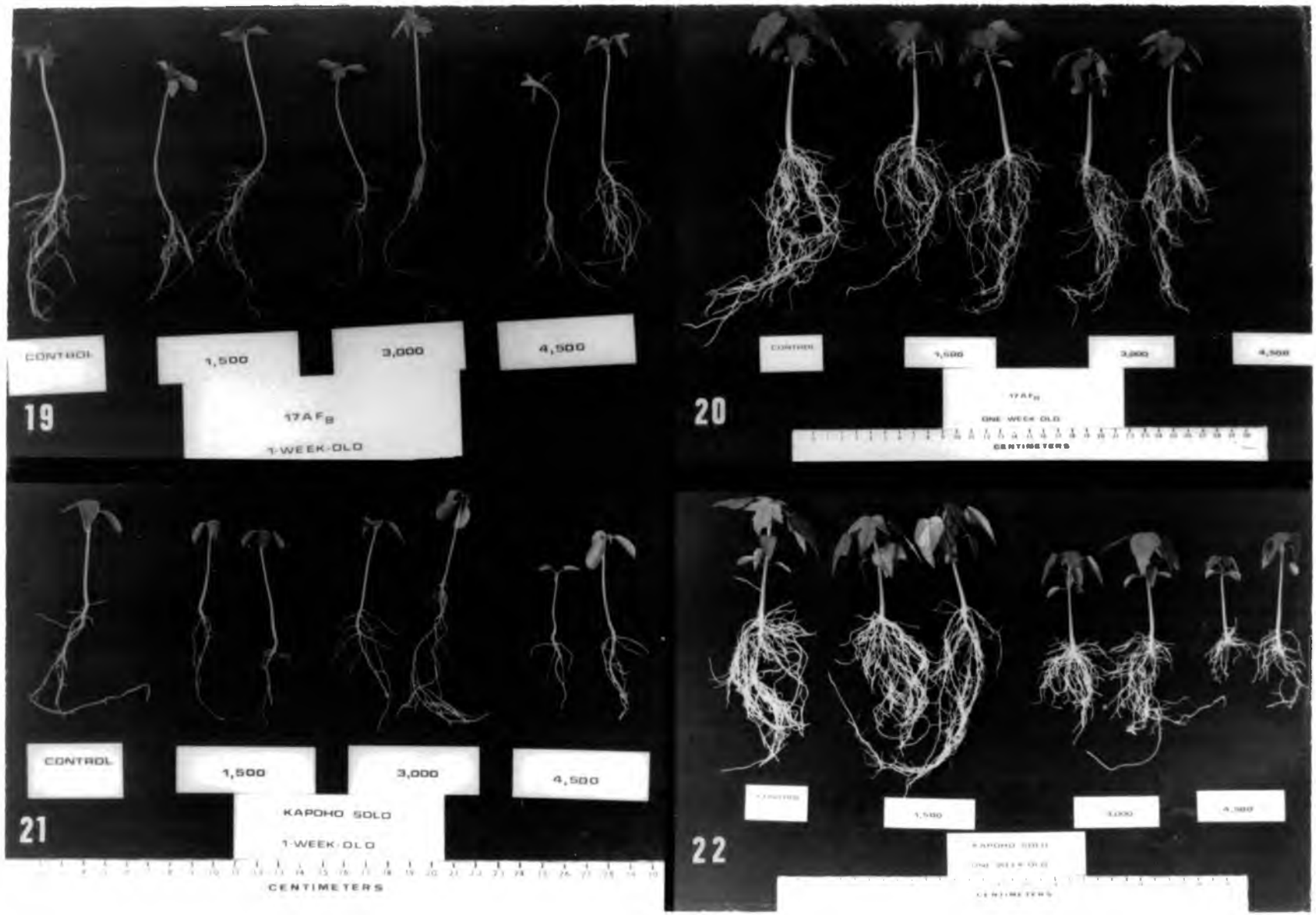

CONTROL
1,500
3,000
4,500
19
17AF8
1-WEEK-OLD
20
ONE WEEK OLD
CENTIMETERS
CONTROL
1,500
3,000
4,500
21
KAPOHO SOLO
1-WEEK-OLD
CENTIMETERS
22

PLATE II (continued)

Figure 23. One-week old seedlings of 'Waimanalo'-23, one week after inoculation

Figure 24. 1-week old seedlings of 'Waimanalo'-23, one month after inoculation

Figure 25. 2-week old seedlings of 'Higgins', one week after inoculation

Figure 26. 2-week old seedlings of 'Higgins', one month after inoculation

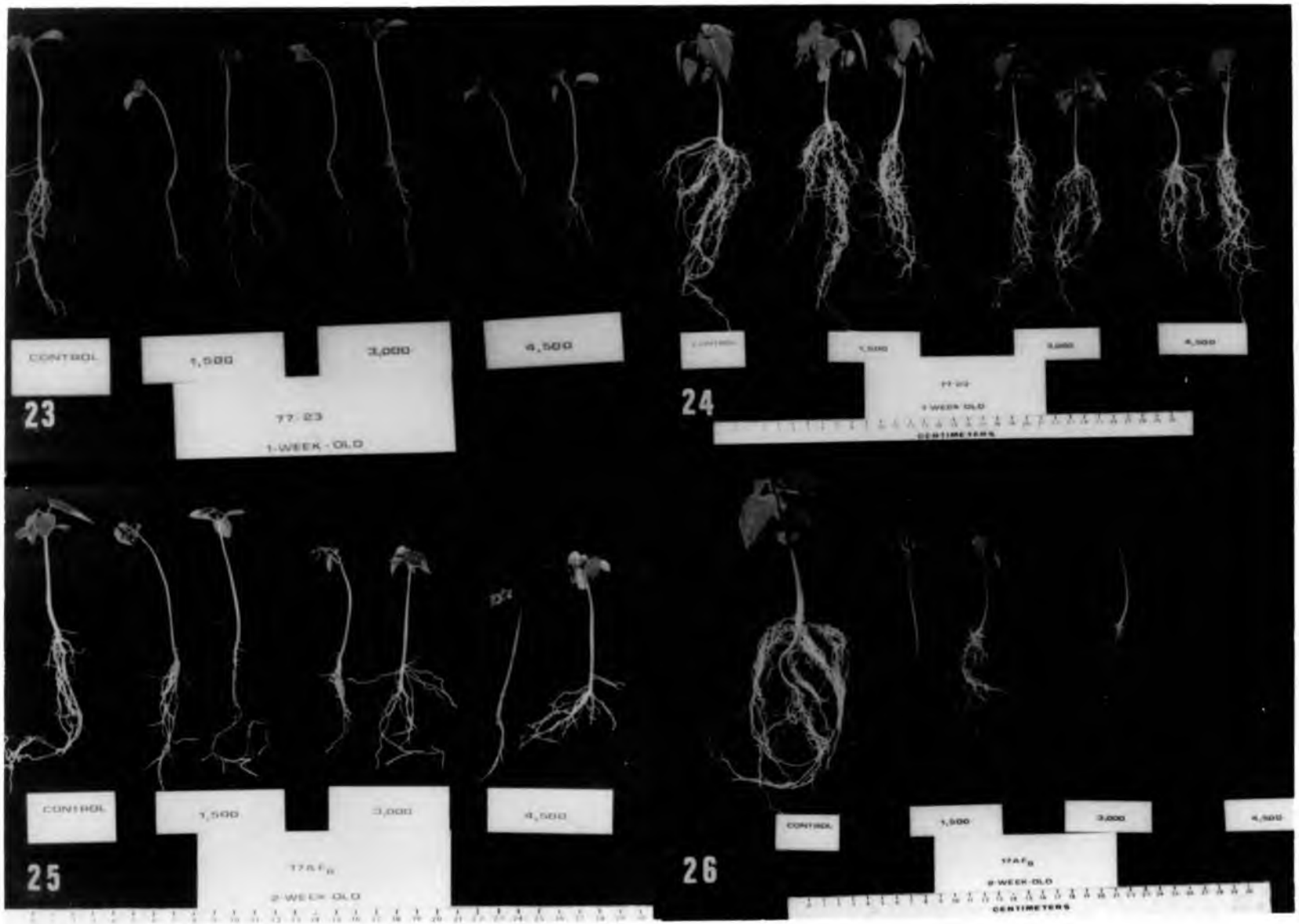

CONTROL
1,500
3,000
4,500
23
77-23
1-WEEK-OLD
24
1-WEEK-OLD
CENTIMETERS
CONTROL
1,500
3,000
4,500
25
2-WEEK-OLD
26
2-WEEK-OLD
CENTIMETERS

PLATE II (continued)

Figure 27. 2-week old seedlings of 'Kapoho' Solo, one week after inoculation

Figure 28. 2-week old seedlings of 'Kapoho' Solo, one month after inoculation

Figure 29. 2-week old seedlings of 'Waimanalo'-23, one week after inoculation

Figure 30. 2-week old seedlings of 'Waimanalo'-23, one month after inoculation


27
CONTROL
1,500
3,000
4,500
KAPOHO SOLO
2-WEEK-OLD
CENTIMETERS
28
CONTROL
1,500
3,000
4,500
KAPOHO SOLO
2-WEEK-OLD
29
CONTROL
1,500
4,500
77-23
2-WEEK-OLD
CENTIMETERS
30
CONTROL
1,500
3,000
4,500
77-23
CENTIMETERS

'Higgins' which had been demonstrated to be a susceptible cultivar.

Percent mortality of 1-month old seedlings of ten papaya lines inoculated with different concentrations of P. palmivora showed 'Waimanalo'-23 (Fig. 31) to be the most resistant line at all concentrations of inoculum tested (Table 7). 'Sunrise' Solo (Fig. 32) and Line 40 showed some resistance. Their means differed significantly and both were significantly more resistant than all other lines tested. Susceptible lines included 'Higgins' (Figs. 10 and 11), 72-8(71-4)T OP (Fig. 33), HAES 8018 (Fig. 34), 41, 25, L-3-6, and L-15-14.

The results of this experiment showed an increase in percent mortality as the concentration of inoculum increased. At low levels of inoculum, resistant or moderately resistant lines such as 'Waimanalo'-23 and 'Sunrise' Solo, showed a low level of mortality, whereas susceptible lines like 'Higgins' had up to 80% mortality. Susceptible lines such as 25, L-3-6, and L-15-14 showed 100% mortality at the lowest concentration of inoculum tested. When 2-month old seedlings of 11 papaya lines were inoculated with the same three concentrations of inoculum, a similar response was observed as shown in Table 8. 'Waimanalo'-23 (Fig. 35) and Line 40 showed the highest level of resistance. Lines 72-8(71-4)T OP (Fig. 37) and 41 (Fig. 38) were moderately resistant at this age, and the rest of the lines were found highly susceptible, with 'Higgins' (Figs. 10 and 11) included in this group. In general, the papaya lines had lower mortality at 2 months of age than when they were

TABLE 7. -- Mean percent mortality of one month old papaya seedlings recorded 1 month after inoculation with P. palmivora

Lines	Concentrations sp/pl. Control	4,500	9,000	18,000	Line mean	
Waimanalo-23	0	0	15	20	8.8	
Sunrise Solo	0	0	55	100	38.8	
40	0	38	80	96	53.2	
72-8(7104) T OP	0	70	80	100	62.5	
HAES 8018	0	67	86	100	63.1	K ratio t (0.05) 8.5%
Higgins	0	80	100	100	70.0	
41	0	96	100	100	74.0	
25	0	100	100	100	75.0	
L-3-6	0	100	100	100	75.0	
L-15-14	0	100	100	100	75.0	
Concen. mean	0	65	82	92		

K ratio t (0.05): 4.4%

LSD (0.05) for 2 lines at same concentration: 16.9%
(0.05) for 2 concentrations of same line: 23.4%

PLATE III

Experiment testing three inoculum levels on one-month old seedlings, shown one month after inoculation

Figure 31. 'Waimanalo'-23

Figure 32. 'Sunrise' Solo

Figure 33. 72-8(71-4)T OP

Figure 34. HAES 8018

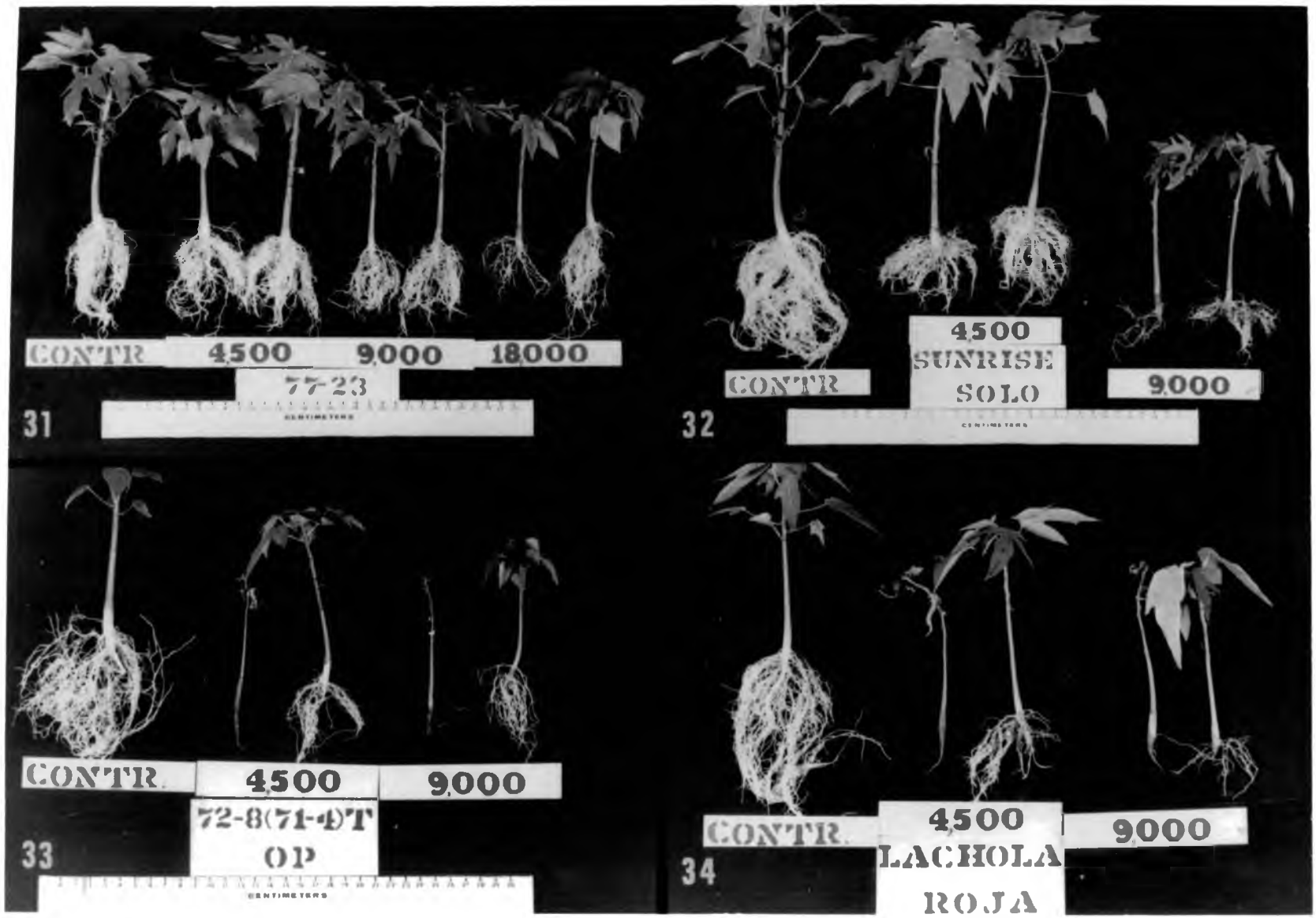

CONTR
4500
9000
18000
77-23
31
4500
SUNRISE
SOLO
CONTR
9000
32
CONTR.
4500
9000
72-8(71-4)T
OP
33
CONTR.
4500
9000
LACHOLA
ROJA
34

TABLE 8. -- Mean percent mortality of two month old papaya seedlings recorded 1 month after inoculation with P. palmivora

Lines	Concentrations sp/pl. Control	4,500	9,000	18,000	Line mean	
Waimanalo-23	0	12.5	8.4	33.4	13.6	
40	0	14.0	14.5	29.0	14.4	
72-8(71-4) T OP	0	8.3	37.5	95.9	35.4	
41	0	54.2	70.9	75.0	50.0	K-ratio
$45\text{-}T_{22}$	0	41.7	79.2	91.7	53.1	t (0.05)
Sunrise Solo	0	50.0	87.5	100.0	59.4	14.7%
25	0	58.3	91.7	100.0	62.5	
Higgins	0	75.0	80.0	100.0	63.8	
L-3-6	0	75.0	81.5	100.0	64.1	
HAES 8022	0	80.0	90.0	95.0	66.3	
L-15-14	0	81.5	94.0	100.0	68.9	
Concen. means	0	50.0	66.8	83.6		
	K-ratio t (0.05): 19.3%					

LSD (0.05) for two lines at some concentration: 16.9%
(0.05) for two concentrations of same line: 44.0%

PLATE IV

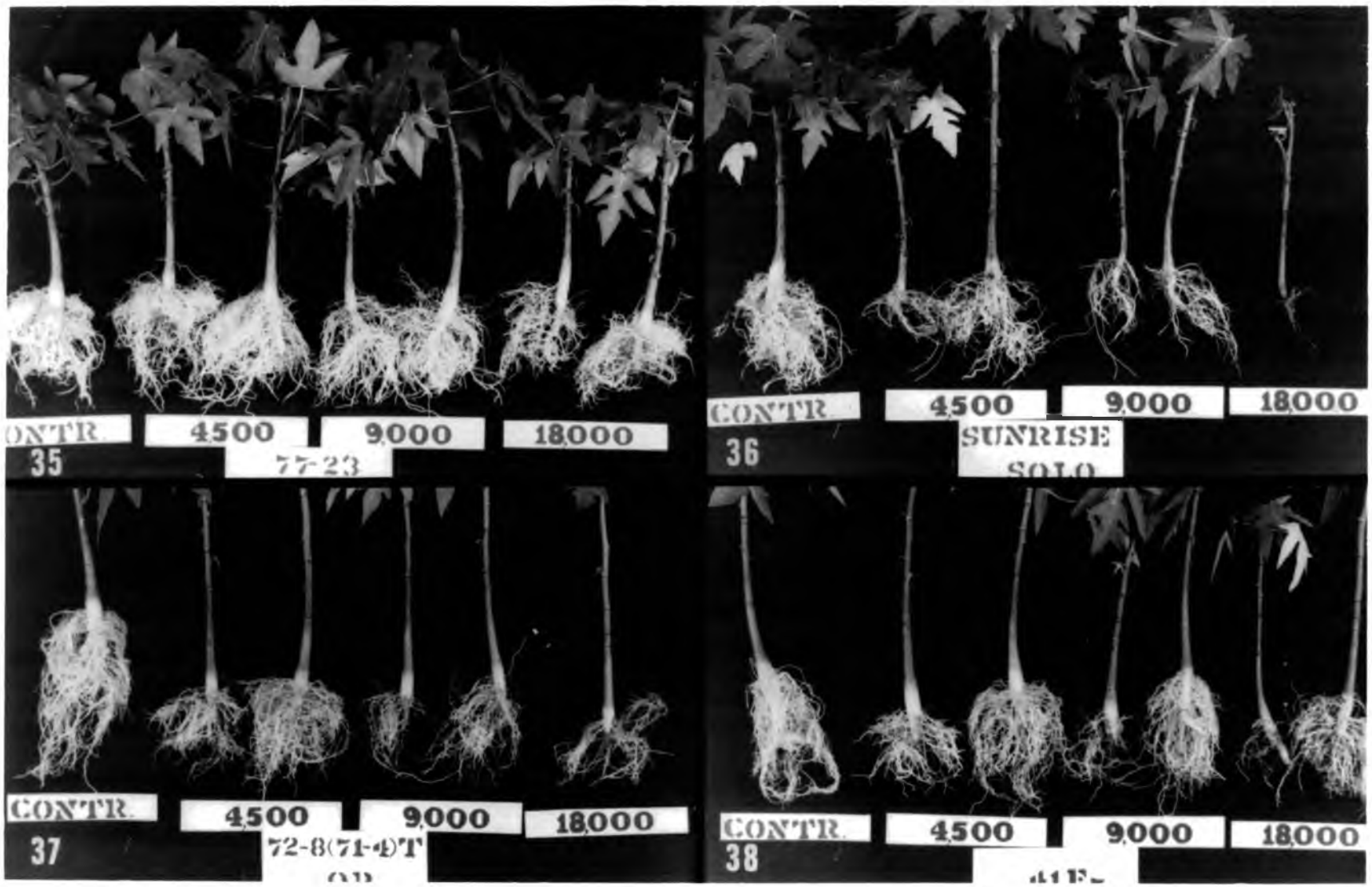
Experiment testing three inoculum levels on 2-month old seedlings, shown one month after inoculation

Figure 35. 'Waimanalo'-23

Figure 36. 'Sunrise' Solo

Figure 37. 72-8(71-4)T OP

Figure 38. Line 41


ONTR.
4500
9,000
18,000
35
77-23
CONTR.
4500
9,000
18,000
36
SUNRISE
SOLO
CONTR.
4500
9,000
18,000
37
72-8(71-4)T
CONTR.
4500
9,000
18,000
38

1-month old as shown by comparison of data from Tables 7 and 8. An exception was noted in that 'Sunrise' Solo (Fig. 36) showed higher mortality at two months of age than at 1 month but this could have been due to sampling error. The trend for most lines to become increasingly resistant with increasing age confirms previous results with 'Higgins' (Fig. 5) and also confirms reports of previous workers.

It should be pointed out that only 2-month old seedlings of 'Waimanalo'-23 and Line 40, and 1-month old seedlings of 'Waimanalo'-23 did not show significant differences in percent mortality with increasing levels of inoculum tested, although there was a trend toward higher mortality. Since higher concentrations were not tested, it is not known whether or not the mechanism of resistance could be overcome with higher concentrations of inoculum than those tested. In moderately resistant lines, however, the mechanism is overwhelmed. This has been reported also in cacao (Amponsah et al., 1973).

These two experiments demonstrated the usefulness of the method of inoculation developed, as well as the presence of resistance in 'Solo' type papayas to root rot caused by P. palmivora.

5. Correlation between percent mortality recorded in field plantings and percent mortality in papaya lines inoculated with the greenhouse method.

To confirm the reliability of the inoculation method developed for papaya, seedlings of the lines listed in Table 2 were inoculated when 1-month old with an inoculum containing

5,250 sporangia/plant. The results shown in Table 9 are mean percent mortality and disease ratings of ten replicates recorded one month after inoculation. Table 9 also shows the correlation coefficient between percent mortality in the field and mean percent mortality in the greenhouse.

Data shown in Table 9 indicate that both variables rank the papaya lines into 3 groups. Resistant lines are: 'Waimanalo'-23, 'Waimanalo'-24, Line 8 and Line 40; moderately resistant lines are: 'Kapoho' Solo, and 45-T_{22}; susceptible lines are: 'Higgins'. The correlation coefficient was positive and highly significant providing supporting evidence for the reliability of the greenhouse inoculation method. Separating all papaya lines tested into resistant and susceptible lines supported the results of field testing. The percent mortality for 'Higgins' (Table 9) is in agreement with the predicted value of 70% calculated from the regression of percent mortality on concentration of inoculum (Fig. 5). These results further support the validity of the prediction and the reliability of the differences in level of resistance found between lines. Knutson and Eide (1961) stated that the best way to assess differences in resistance is to inoculate the test varieties with an equal amount of inoculum. This was the method used in this study.

GENETICS OF ROOT ROT RESISTANCE IN PAPAYA

The mean percent mortality recorded in the five parental lines and the ten F_1 hybrids obtained from them by using the half diallel mating

TABLE 9. -- Mean percent mortality and disease ratings of one month old papaya seedlings recorded one month after inoculation with 5,250 sp/plant of *P. palmivora*

Lines	% mortality		Disease ratings[x]
	Transformed arc sin $\sqrt{\%}$	Retransformed %	
Waimanalo-23	12.28	4.5	3.7
Waimanalo-24	14.79	6.5	3.8
Line 8	19.32	10.9	3.5
40	19.33	11.0	3.4
Kapoho Solo	30.74	26.1	2.9
$45\text{-}T_{22}$	34.98	32.9	2.5
Higgins	54.63	66.5	1.7
CV %	39.60		17.3
K-ratio t (0.05)	8.1		0.4

[x]Rating scale measured from 1 = dead plant, to 5 = healthy vigorous plant

Correlation between field mortality and greenhouse mortality:
r = 0.9355**

system are shown in Table 10.

The parental lines ranged from 4.5% to 66.5% mortality and the full-sib families ranged from 6.1% in 'Waimanalo'-23 x 'Waimanalo'-24 a cross of resistant lines, to 34.4% in 45-T_{22} x 'Higgins' a cross between a moderately resistant and a susceptible line.

The analysis of variance of percent mortality transformed to angles by angle = arc sin $\sqrt{\%}$ is presented in Table 11 following the methods of Hayman (1954a, 1954b, and 1960); Jones (1965) and Mather and Jinks (1971). The analysis shows significant additive and dominance variance noted as items a and b respectively, when tested against their own block interaction mean square or against the pooled error mean square. Dominance variance was significant at the 5% level when tested against the pooled error mean square. In accordance with Mather and Jinks' (1971) theory, this test failed to show genotype x environmental interaction. When item b was partitioned into b_1, b_2, and b_3, only b_2 was found to be highly significant, indicating assymetry of gene distribution. This suggests that positive and negative alleles are in different proportions in the parents (Mather and Jinks, 1971; Hayman, 1954b).

The next step in the analysis was to test whether or not an additive-dominance model applies to the data, under the assumptions of independent gene distribution between the parents, homozygosity of the parental lines, diploid segregation and no multiple allelism present. To test these hypotheses an analysis of variance of the W_r- V_r values over arrays was performed and is shown in Table 12. W_r is the covariance between the parents and their progeny in the r^{th} array and V_r is the variance of the r^{th} array. Lack of significance of the mean

TABLE 10. -- Mean percent retransformed mortality obtained in a 5 x 5 half diallel by inoculation with a uniform concentration of *P. palmivora*[x]

Seed parent	Pollen parent					Mean progenies
	40	Waimanalo 23	45-T_{22}	Waimanalo 24	Higgins	
40	11.0	8.8	12.5	8.9	18.2	11.8
Waimanalo-23		4.5	18.9	6.1	22.8	13.4
45-T_{22}			32.9	26.9	34.4	22.6
Waimanalo-24				6.5	20.5	14.4
Higgins					66.5	23.5

K-ratio t: (0.05) = 10.5%; (0.01) = 13.6%

[x]Overall means based on 10 replications. Five of them having 50 plants per treatment, and the other five with 25 plants per treatment. Both experiments were pooled for analysis.

TABLE 11. -- Mean squares and degrees of freedom of transformed percent mortality in papaya obtained in a 5 x 5 half diallel

Source of variation	df	ms	F vs own interaction	F vs pooled interaction
a	4	3,666.50	23.69**	35.92**
(b)	(10)	232.67	2.87**	2.28*
b_1	1	287.47	2.91 ns	2.82 ns
b_2	4	391.11	6.38**	3.83**
b_3	5	94.87	1.02 ns	0.93 ns
Blocks	9	1,795.33		17.59**
B x a	36	154.80		
(B x b)	(90)	80.97		
B x b_1	9	98.66		
B x b_2	36	61.34		
B x b_3	45	93.13		
Error	126	102.06		

TABLE 12. -- Analysis of variance of $W_r - V_r$ for transformed percent mortality in a 5 x 5 half diallel in papaya

Source of variation	df	ms	F
Arrays	4	3,736.42	0.57 ns
Blocks	9	26,572.98	4.05 **
Error	36	6,524.20	

ns: no significance
**: significant at 1% level of probability

square for arrays in Table 12 demonstrates the constancy of $W_r - V_r$ values over arrays and suggests the validity of the hypotheses proposed and that an additive-dominance model is an acceptable interpretation of the data.

The V_r, W_r graphic analysis was also done since it provides information on the relative amounts of dominant and recessive alleles in the parents, and degree of dominance in the population analyzed (Fig. 39). The regression coefficient of V_r on W_r provides another way to test validity of the hypotheses. The regression coefficient was shown to be different from zero but not different from one, indicating the validity of the hypotheses (Hayman, 1954b).

The fact that the slope of the regression line is equal to one indicates that dominance is present supporting the results of the analysis of variance (Table 11). The regression line, however, crosses the W_r axis above the origin. This strongly suggests that dominance is only partial (Hayman 1954b; Mather and Jinks, 1971). From the position of the parental arrays relative to the regression line the parental lines may be ranked as follows in descending order relation to the probable number of dominant alleles they contain: Line 40, 45-T_{22}, 'Waimanalo'-23, 'Waimanalo'-24, and 'Higgins'.

Components of variation and standard errors, as well as the estimated heritability values and other estimated parameters are shown in Table 13. Most of the components of variation were significantly different from zero. $\hat{F}$ showed significance only between 5 and 10% level of probability, and $\hat{h}^2$ failed to show significance. $\hat{h}^2$ tests b_1 which did not show significance (Table 11), indicating agreement between both analyses. The estimates of narrow sense heritability of resistance

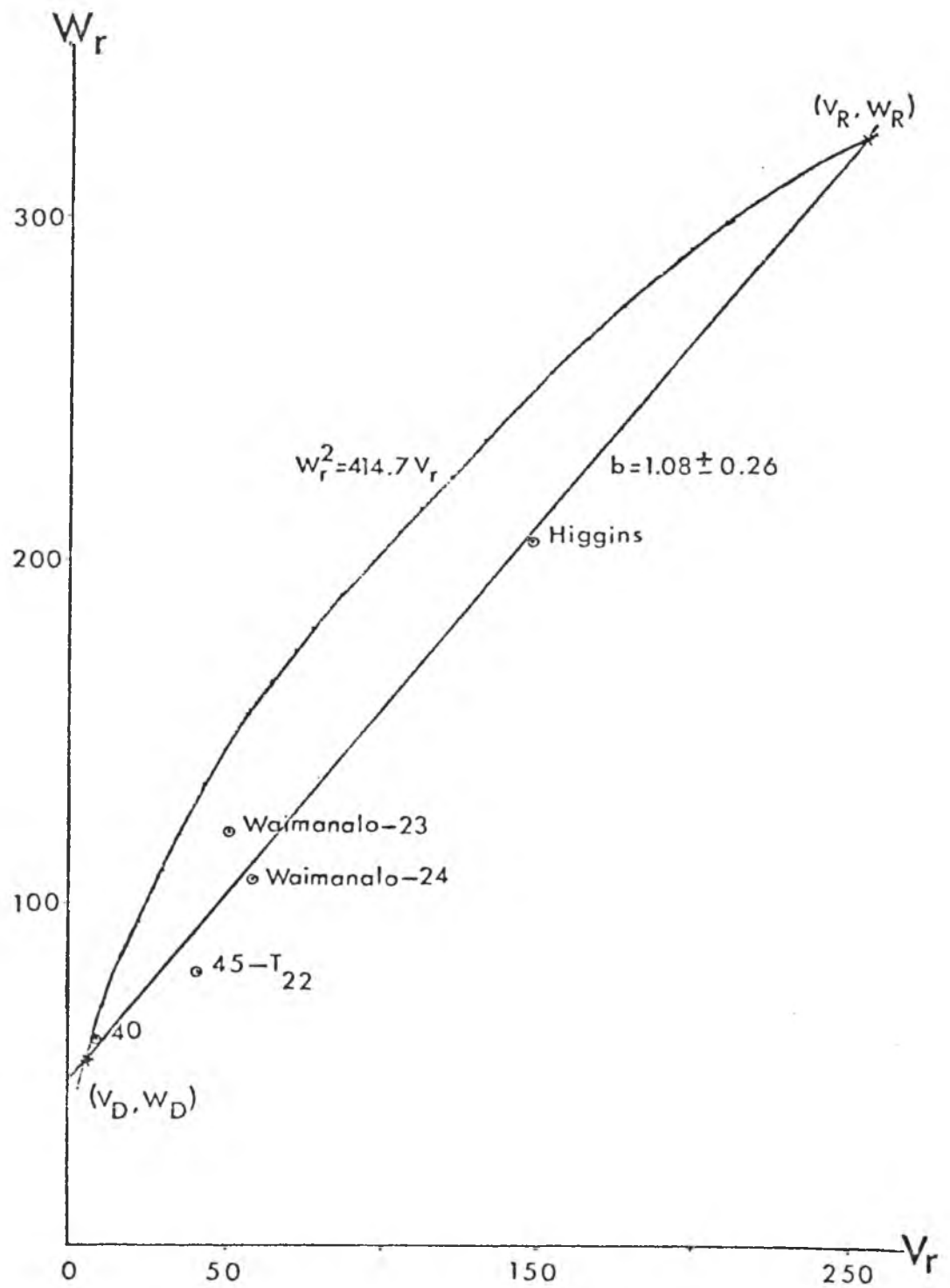


Fig. 39 (V_r, W_r) graph of transformed percent mortality in a 5 x 5 half diallel of papaya lines

TABLE 13. -- Components of genetic and environmental variation and heritability estimates of transformed percent mortality as an index of root rot resistance in papaya

Component of variation	Mean	+/-	Standard error	Probability
$\hat{D}$	312.71		22.20 **	$P < 0.001$
$\hat{F}$	107.34		55.45	$0.05 < P < 0.10$
$\hat{H}_1$	132.72		59.94 *	$P < 0.05$
$\hat{H}_2$	137.61		54.37 *	$P < 0.05$
$\hat{h}^2$	9.88		36.70 ns	$P > 0.50$
$\hat{E}$	102.06		9.06 **	$P < 0.01$
narrow heritability =	0.42			
broad heritability =	0.57			

Mean degree of dominance $\sqrt{\hat{H}_1 / \hat{D}} = 0.65$

Proportion of genes with positive and negative effects in the parents

$$\hat{H}_2 / 4\hat{H}_1 = 0.26$$

Proportion of dominant and recessive genes in the parents:

$$\frac{\sqrt{4\hat{D}\ \hat{H}_1 + \hat{F}}}{\sqrt{4D\ H_1 - F}} = 1.72$$

to P. palmivora root rot in papaya indicate that considerable additive genetic variation is present. Dominance variance is also present but in smaller amount and exists only as partial dominance as indicated by the estimated mean degree of dominance shown in Table 13, which is also in agreement with the regression line shown in Fig. 39. The estimate of the proportion of genes with positive and negative effects in the parents, suggests that frequency of alleles controlling resistance to root rot is very close to the condition $p = q = 0.5$. This result however, does not agree with that in the analysis of variance in Table 11, where the statistical significance of b_2 suggests gene assymetry.

In Table 13, the proportion of dominant and recessive genes in the parents shows the presence of assymetry which can result from over estimation. Hayman (1954b) indicates that residual heterozygosity in the parents over estimates F, exaggerates assymetry of gene distribution and the proportion of dominant to recessive alleles. If F were over estimated in this study this would further confirm that it is actually no significant. This would also suggest that gene assymetry is an over estimation. Mean degree of dominance and the proportion of positive and negative alleles are under estimated if there is residual heterozygosity in the parents. The detection of only partial dominance suggests that dominance has been under estimated in this study. The proportion of positive and negative alleles, however, shows not to be under estimated. Presence of residual heterozygosity, however, is not supported by the analysis of $W_r - V_r$ (Table 12) since the mean square of arrays was not significant and did not reject validity of the postulated hypotheses.

On the other hand, the change of scale from percent mortality to

angles may have affected interpretation of the data as suggested by Falconer (1960) and Mather and Jinks (1971).

The possibility of using either percent mortality, which usually shows heterogeneity of variances invalidating analysis of variance (Snedecor and Cochran, 1967), or that transforming the data could complicate the genetic interpretation, suggests the possibility of using disease ratings in the genetic analysis to avoid such complications.

Means of disease ratings for the 5 parental lines and 10 F_1 hybrids of the half diallel are presented in Table 14. Disease ratings ranged from 1.7 in 'Higgins' the most susceptible parent, to 3.8 in 'Waimanalo'-24. The full-sib families ranged from 2.4 in the cross 45-T_{22} x 'Higgins', to 3.9 in the cross between the two sublines of 'Waimanalo'. These results are compatible with those for percent mortality shown in Table 10.

The analysis of variance for disease ratings is shown in Table 15. Contrary to results obtained when percent mortality was analyzed (Table 11), this analysis failed to show significance for dominance variance or any one of its components b_1, b_2 or b_3. It was, however, effective in detecting highly significant additive variation.

Additive genetic variance was highly significant when tested against its own interaction and against the pooled block interaction. This agrees with the previous analysis of percent mortality and indicates lack of genotype x environment interaction (Mather and Jinks, 1971).

When disease ratings were analyzed dominance variation was not detected. When transformed percent mortality was analyzed however,

TABLE 14. -- Mean disease rating obtained in a 5 x 5 half diallel by inoculation with a uniform concentration of P. palmivora

Seed parent	Pollen parent					Mean progenies
	40	Waimanalo 23	45-T_{22}	Waimanalo 24	Higgins	
40	3.4	3.4	3.1	3.5	2.8	3.2
Waimanalo-23		3.7	2.9	3.9	2.9	3.3
45-T_{22}			2.5	2.8	2.4	2.8
Waimanalo-24				3.8	3.0	3.3
Higgins					1.7	2.8

K-ratio t: (0.05) = 0.43; (0.01) = 0.55

TABLE 15. -- Mean squares and degrees of freedom of disease ratings in papaya obtained in a 5 x 5 half diallel

Source of variation	df	ms	F vs own interaction	F vs pooled interaction
a	4	11.12	34.32 **	41.73 **
(b)	(10)	0.39	1.61 ns	1.47 ns
b_1	1	0.07	0.50 ns	0.27 ns
b_2	4	0.47	2.48 ns	1.75 ns
b_3	5	0.40	1.30 ns	1.50 ns
Blocks	9	3.07		11.52 **
B x a	36	0.32		
(B x b)	(90)	0.24		
B x b_1	9	0.14		
B x b_2	36	0.19		
B x b_3	45	0.31		
Block interaction	126	0.27		

significant dominance variation was found. Both scales were assumed to be indices of resistance to root rot. Detection of dominance in analyzing percent mortality may have been due to the change in scale (angle = arc sin $\sqrt{\%}$), rather than to residual heterozygosity in the parents. Many researchers have preferred disease rating scales to conduct analysis of heritability (Verhalen et al., 1971; Nelson and Scott, 1973; Lu et al., 1973).

The lack of significance of $W_r - V_r$ mean square over arrays shown in Table 16 indicates the validity of the hypotheses. The plotting of the V_r, W_r values (Fig. 40) shows that regression line passes through the point of origin, although it is not significantly different from zero which would indicate that dominance is not present, which agrees with the analysis of variance shown in Table 15.

According to Hayman (1954b), if there is no dominance, the regression line should be tangent to the limiting parabola, and all points (V_r,W_r) should coincide at the point of contact $(\frac{1}{4}D,\frac{1}{2}D)$. In this case the point of contact lies inside the parabola (Fig. 40). Since the analysis of $W_r - V_r$ did not show any reason to reject the validity of the hypotheses but the (V_r,W_r) graph showed some disagreements, it is probable that there is some correlation of the gene distribution. It is also likely that some non-allelic interaction is present as a consequence, i.e. complementary gene action inflates the degree of dominance (Hayman, 1954b), which seems to be the case here. If this were the case, $\hat{H}_1$ should be greater than 0.

Estimates of the components of variation, heritability, and other parameter estimates are shown in Table 17. The components of variation that showed significance for percent mortality (Table 13) and for

TABLE 16. -- Analysis of variance of $W_r - V_r$ for disease rating scale in a 5 x 5 half diallel in papaya

Source of variation	df	ms	F
Arrays	4	0.0031	0.06 ns
Blocks	9	0.3847	7.88 **
Error	36	0.0488	

TABLE 17. -- Components of genetic and environmental variation and heritability estimates of disease ratings as an index of root rot resistance in papaya

Component of variation	Mean	+ -	Standard error	Probability
$\hat{D}$	0.7631		0.0514 **	P < 0.01
$\hat{F}$	0.1225		0.1284 ns	
$\hat{H}_1$	0.3933		0.1387 **	P < 0.01
$\hat{H}_2$	0.3800		0.1259 **	P < 0.01
$\hat{h}^2$	0.0251		0.0849 ns	
$\hat{E}$	0.2664		0.0210 **	P < 0.01
narrow heritability =	0.47			
broad heritability =	0.61			

Mean degree of dominance = 0.72

Proportion of genes with positive and negative effects in the parents = 0.24

Proportion of dominant and recessive genes in the parents = 1.25

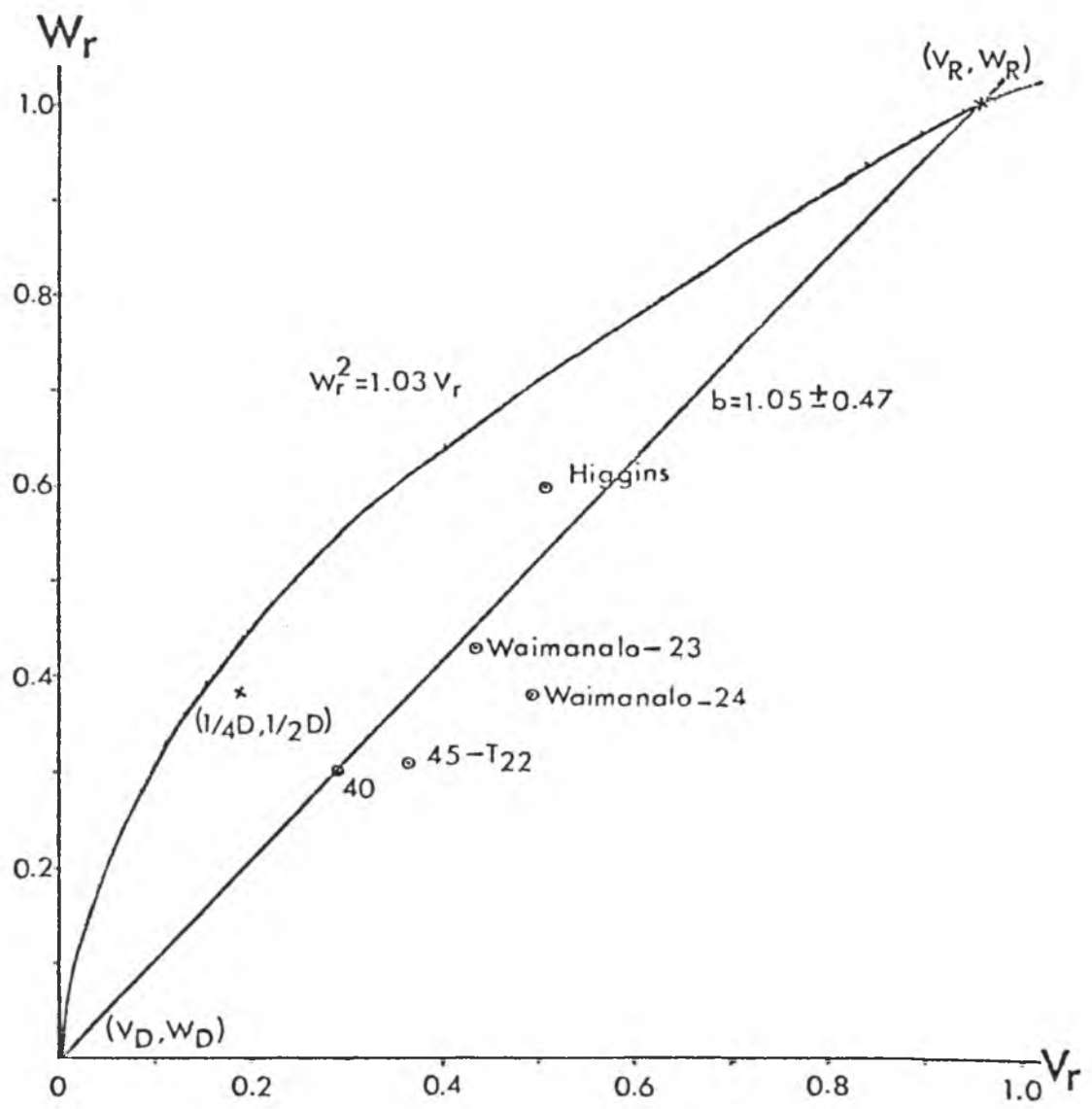


Fig. 40 (V_r, W_r) graph of disease ratings in a 5 x 5 half diallel of papaya lines

disease ratings (Table 17) were $\hat{D}$, $\hat{H}_1$ and $\hat{H}_2$. Narrow sense and broad sense heritability estimates were very similar for both scales, disease ratings and percent mortality. This further confirms that additive genetic variance is present and in both cases considerably high.

Some doubt remains regarding the estimate of dominance. The mean degree of dominance in Table 17 suggests partial dominance. Although it was neither detected by the analysis of variance (Table 15), nor shown in the V_r, W_r graph (Fig. 40). The value of $\hat{H}_1$ was significantly different from zero which may be due to the presence of non-allelic interaction in the form of complementary gene action (Hayman, 1954b).

The proportion of genes with positive and negative effects in the parents was 0.24, which is in close agreement to the result shown by percent mortality. This suggests that gene frequency for the considered character is very close to the condition $p = q = 0.5$. This is logical since the parent lines were assumed to be homozygous and the only progenies grown were the F_1 hybrids. This is supported by the proportion of dominant and recessive alleles in the parents which was 1.25, with an $\hat{F}$ value not significantly different from zero so that the proportion is almost 1.00. These two proportions would support the absence of gene assymetry, also suggested by lack of significance of b_2 (Table 15), and the fact that $\hat{H}_1 = \hat{H}_2$ (Table 17).

The interpretation of dominance variation is still obscure. Although the validity of the hypotheses was not rejected by the $W_r - V_r$ analysis there are contradictions from the estimated mean degree of dominance. It might have happened that a balanced failure occurred since $W_r - V_r$ values are often correlated. This has been pointed out by Hayman (1954b). To further clarify this situation it is necessary to

obtain the F_2 or backcrosses or both types of generations. This problem does not however invalidate the estimates of the components of variation and it has no effect on estimation of narrow heritability (Hayman, 1954b) which is the most important estimate in this study.

From this accumulated information it appears that significant additive genetic variation exists for root rot resistance to P. palmivora in the papaya lines included in this study. It also seems that nonallelic interaction may be involved but further studies would be needed to assess its importance.

Other analysis are available for the half diallel, and in order to assess the importance of the estimations of additive variation obtained by using Hayman's analysis, the data were analyzed using Griffing's method 2, model II (1956b).

Analysis of variance for transformed percent mortality is shown in Table 18, and that for the rating scale in Table 19. Components of variance and heritabilities for both variables are shown in Table 20. Data shown in Tables 18 and 19 indicate that the mean squares for general combining ability and specific combining ability are exactly the same as in Hayman's analysis of mean squares of a and b items respectively shown in Tables 11 and 15. It should also be noted that Griffing's analysis detects significant dominance variance for transformed percent mortality as did Hayman's as is shown in Tables 18 and 11. When the disease rating scale was analyzed by both methods of analysis dominance variation was not detected (Tables 15 and 19). As mentioned previously it is probable that the change in scale from percent mortality into angles changes the genetic interpretation (Falconer, 1960; Mather and Jinks, 1971), inasmuch as the data in

TABLE 18. -- Analysis of variance for transformed percent mortality of a 5 x 5 half diallel. Griffing's method 2, model II

Source of variation	df	ms	F
Blocks	9	1,795.33	17.59 **
Crosses	(14)	1,213.73	11.89 **
GCA	4	3,666.50	35.92 **
SCA	10	232.63	2.28 *
Error	126	102.06	

TABLE 19. -- Analysis of variance of disease ratings for a 5 x 5 half diallel. Griffing's method 2, model II

Source of variation	df	ms	F
Blocks	9	3.07	11.37 **
Crosses	(14)	3.46	12.81 **
GCA	4	11.12	41.19 **
SCA	10	0.39	1.46 ns
Error	126	0.27	

TABLE 20. -- Components of variation and heritabilities for transformed percent mortality and disease ratings as indices for root rot resistance in papaya, in a 5 x 5 half diallel

Component of variation	Transformed % mortality arc sin $\sqrt{\%}$	Disease rating
σ^2_g	490.6 $\pm$ 370.7	1.5 $\pm$ 1.1
σ^2_s	13.1 $\pm$ 104.8	0.01 $\pm$ 0.18
σ^2_e	102.1 $\pm$ 12.9	0.27 $\pm$ 0.03
additive variance	981.1	3.06
dominance variance	13.1	0.01
genetic variance	994.2	3.08
phenotypic variance	1096.1	6.25
Narrow sense heritability	0.89	0.916
Broad sense heritability	0.91	0.919

Table 10 fail to show dominance.

Heritability estimates obtained for both variables using Griffing's method of analysis, resulted in very high estimates (Table 20), which were almost twice as high as the ones obtained with Hayman's analysis (Tables 13 and 17). Both analyses, however, agree in demonstrating that a highly significant amount of additive genetic variation for resistance to root rot caused by P. palmivora is present in the population studied. The difference in the heritability values must be due to the different approaches in the two methods used. Further studies should be carried out by obtaining the F_2 and backcross generations of the 5 x 5 half diallel. This would allow the use of other methods of analysis, such as the generation mean analysis (Mather and Jinks, 1971) or the analysis of the half diallel used in this study. With later generations it would also allow the inclusion of epistasis in the model (Mather and Jinks, 1971). If selection is carried out for several generations, realized heritability can be calculated (Falconer, 1960). If the use of model II of Griffing's (1956b) is questionable because the parent lines were selected rather than picked at random, the analysis proposed by Kuehl et al. (1968) may be used if later generations are obtained by random mating of the parental lines. These authors indicate that the condition of chosing the parental lines at random is difficult to meet so they developed a model that uses a fixed set of inbred lines mated at random following a diallel mating system to give information on additive, dominant, and epistatic variances. This information can then be applied to the population derived from the diallel.

The artificial inoculation conditions under which the estimates of genetic variances and heritability for root rot resistance in papaya

were determined appears to be a valid one since the primary objective was to determine the presence of genetic resistance to root rot and in these cases, only one environment, that of a high disease incidence, is needed to obtain valid estimates of genetic variance, as has been indicated by Dudley and Moll (1969). It should be stressed that screening of genetic materials should be carried out under the same conditions determined in this study unless an increase of the selection intensity is desired. If selection intensity needs to be increased, concentrations of inoculum should be tested.

In a papaya breeding program such as in Hawaii where the aim is to use homozygous lines as cultivars, the narrow heritability estimate is the one considered more important. Selection among homozygous lines or populations derived from them can be carried out and be of immediate commercial use as has been suggested for similar cases by Dudley and Moll (1969).

An objective of this study as stated previously was to determine heritability, so that a choice of selection procedure could be made to obtain faster genetic advance. The heritability estimates have been already presented in the previous section and now consideration will be given to the intensity of selection.

The intensity of selection depends only on the proportion of the population included in the selected group (Falconer, 1960). In this study by artificial inoculation with P. palmivora to the papaya progenies obtained from the half diallel, a calculation was made of the percent selectable plants obtained one month after inoculation. This percent of selectable plants was defined by Nakasone and Aragaki (1973), as those plants with a disease rating of 4 or above. This selected group is

actually the most resistant group of plants. Results obtained with this variable are presented in Table 21, which includes two control lines as well as the families obtained from the 5 x 5 half diallel.

The analysis of variance for percent selectable plants showed highly significant differences between lines and F_1 hybrids. This variable can provide another index for resistance. The results are similar to those presented for percent mortality and disease ratings. The classification of lines according to resistance is: resistant: 'Waimanalo'-23 (Fig. 41), Line 8 (Fig. 42), 'Waimanalo'-24 (Fig. 43), and 40 (Fig. 44); moderately resistant: 'Kapoho' Solo (Fig. 45) and 45-T_{22} (Fig. 47); and susceptible: 'Higgins' (Fig. 46).

Crosses between resistant lines are resistant (Figs. 48, 49 and 50); between resistant and moderately resistant are moderately resistant (Figs. 51, 52, and 53); between resistant and susceptible are moderately resistant (Figs. 54, 55, and 56); and between moderately resistant and susceptible are moderately resistant (Fig. 57).

It should be stressed that selection intensity can be increased by the breeder by simply increasing the concentration of inoculum. A linear cause-effect relationship has been demonstrated previously between concentration of inoculum and percent mortality (Fig. 5). This was shown for the susceptible line, but the same trend was also observed even with resistant lines (Tables 7 and 8). This plus the fact that there are highly significant correlations between the three scales used to rate resistance, namely, % mortality, disease rating, and % selectable plants (Table 21), support the statement that with increasing concentration of inoculum, intensity of selection can be increased, and even by properly adjusting it to obtain a higher percent mortality and

TABLE 21. -- Percent of selectable plants obtained by inoculating 1-month old papaya seedlings with 5,250 sp/plant of P. palmivora, and correlations between three variables used in rating resistance

Lines and F_1 Hybrids	Transformed data arc sin $\sqrt{\%}$	Retransformed % selectable	Correlations r
Waimanalo-24	55.41	67.8	
Waimanalo-23 x 24	55.05	67.2	
Waimanalo-23	51.56	61.4	between %
Line 8	50.35	59.3	mortality
40	47.61	54.6	and % selectable
40 x Waimanalo-23	45.71	51.3	-0.8521 **
40 x Waimanalo-24	44.98	50.0	between %
Waimanalo-24 x Higgins	34.37	31.9	mortality and
Waimanalo-23 x Higgins	34.37	31.9	rating scale:
40 x 45-T_{22}	33.57	30.6	-0.9475 **
Waimanalo-23 x 45-T_{22}	30.30	25.5	
40 x Higgins	28.69	23.0	between %
45-T_{22} x Waimanalo-24	26.92	20.5	selectable and
Kapoho Solo	26.07	19.3	rating scale
45-T_{22}	23.65	16.1	0.9696 **
45-T_{22} x Higgins	21.12	13.0	
Higgins	14.77	6.5	
Means	36.74	35.8	
K-ratio t (0.05)	11.31		

PLATE V

Root rot in papaya lines inoculated with a uniform concentration of *P. palmivora* shown one month after inoculation

Figure 41. 'Waimanalo'-23

Figure 42. Line 8

Figure 43. 'Waimanalo'-24

Figure 44. Line 40

Figure 45. 'Kapoho' Solo

Figure 46. 'Higgins'

Figure 47. Line 45-T_{22}


41
77-23
42
LINE 8
43
77-24
44
40F5
45
KAPOHO
46
17AF8
47
45F6T22

PLATE VI

Root rot in F_1 hybrids obtained in a half diallel inoculated with a uniform concentration of *P. palmivora* shown one month after inoculation

Figure 48. Line 40 x 'Waimanalo'-23

Figure 49. Line 40 x 'Waimanalo'-24

Figure 50. 'Waimanalo'-23 x 'Waimanalo'-24

Figure 51. 'Waimanalo'-23 x Line 45-T_{22}


48
40F5 X 77-23
49
40F5 X 77-24
50
77-23 X 77-24
51
77-23 X 45F6T22

PLATE VI (continued)

Figure 52. Line 40 x Line 45-T_{22}

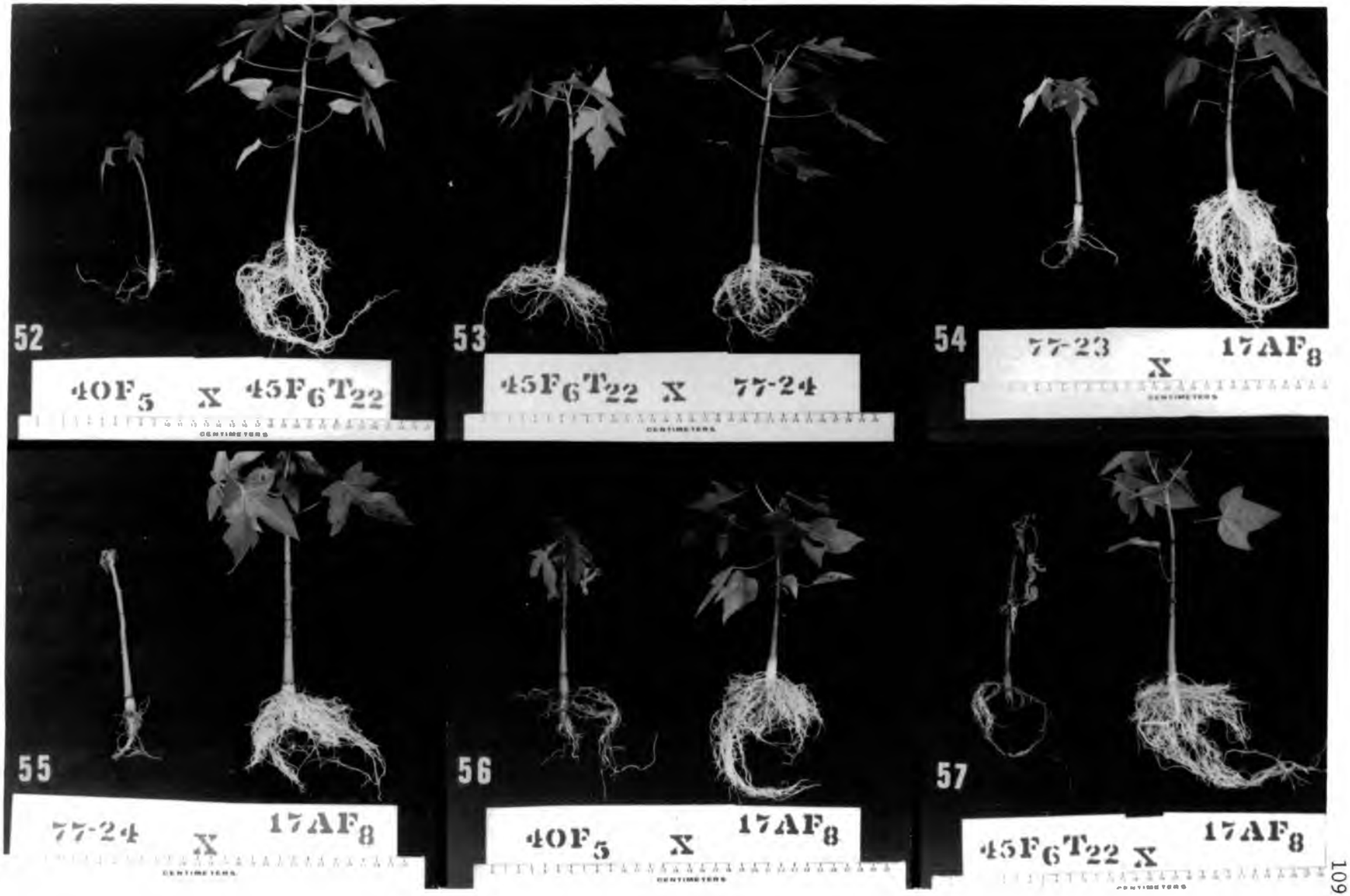
Figure 53. Line 45-T_{22} x 'Waimanalo'-24

Figure 54. 'Waimanalo'-23 x 'Higgins'

Figure 55. 'Waimanalo'-24 x 'Higgins'

Figure 56. Line 40 x 'Higgins'

Figure 57. Line 45-T_{22} x 'Higgins'


52
40F5 X 45F6T22
53
45F6T22 X 77-24
54
77-23 X 17AF8
55
77-24 X 17AF8
56
40F5 X 17AF8
57
45F6T22 X 17AF8

lower percent of selectable plants, so that maximum speed of progress under selection can be attained, as indicated by Falconer (1960).

EFFECT OF P. PALMIVORA ROOT ROT ON PAPAYA SEEDLING GROWTH

Nursery stage

In order to assess the extent to which root rot affected papaya seedling growth several variables were analyzed.

Mean increase in number of nodes per plant were obtained from the inoculated papaya seedlings between the first and second month of growth after germination. Comparisons of each papaya line with the mean of uninoculated papaya seedlings of the same line are shown in Table 22.

There were differences in growth rate within uninoculated lines. 'Waimanalo'-23, and 45-T_{22} as well as the F_1 hybrids having either one of those two lines as parents had the most rapid growth rates. Increase in number of nodes per plant for inoculated seedlings was generally smaller than that for the control seedlings. No significant differences were found in comparing seedlings of the same line under the two conditions, except for line 45-T_{22} and the F_1 hybrids of 45-T_{22} x 'Waimanalo'-24 and 45-T_{22} x 'Higgins'. It should be pointed out that counts of node number were made on the survivors of inoculated plants, so that the sample was made up mostly of resistant survivors. This is probably the reason why lines like 'Higgins', which has been identified as susceptible failed to show difference from the uninoculated set. In general it can be said that in spite of the infection caused to the roots by P. palmivora, surviving papaya seedlings continued to produce new nodes at a rate similar to that of uninoculated plants. An

TABLE 22. -- Comparison of mean increase in number of nodes per plant from inoculated and uninoculated papaya lines during one month's growth

Lines and Hybrids	Outdoors Uninoculated 3 reps.	Greenhouse Inoculated 10 reps.	Difference	$S_{\bar{d}}$	t	t'
Line 8	5.8	5.8	0.0	0.48	0.00 ns	
40	5.9 ± 0.1	5.8 ± 0.9	0.1			0.74 ns
Waimanalo-23	6.7	6.6	0.1	0.54	0.19 ns	
Waimanalo-24	6.3	6.2	0.1	0.57	0.18 ns	
Waimanalo-23 x Waimanalo-24	6.7	6.6	0.1	0.44	0.23 ns	
40 X Waimanalo-23	6.1	5.9	0.2	0.57	0.35 ns	
Waimanalo-23 x Higgins	6.0	5.8	0.2	0.50	0.40 ns	
Higgins	5.9	5.4	0.5	0.35	1.43 ns	
Waimanalo-24 x Higgins	6.1	5.5	0.6	0.47	1.28 ns	
40 x 45-T_{22}	6.1	5.4	0.7	0.57	1.23 ns	
Kapoho Solo	5.3	4.5	0.8	0.56	1.43 ns	
40 x Waimanalo-24	6.6	5.8	0.8	0.49	1.63 ns	
40 x Higgins	6.2	5.4	0.8	0.61	1.31 ns	
Waimanalo-23 x 45-T_{22}	6.7	5.7	1.0	0.56	1.79 ns	
45-T_{22} x Waimanalo-24	6.6	5.6	1.0	0.45	2.22 *	
45-T_{22} x Higgins	6.5	5.3	1.2	0.35	3.43 **	
45-T_{22}	6.8	5.5	1.3	0.50	2.60 *	
Means	6.3	5.7				
K-ratio t (0.05)	0.5	0.6				

exception was however noted in the moderately resistant line 45-T_{22}.

Premature defoliation is a symptom of root rot on papaya (Parris, 1942), and number of leaves lost on inoculated plants during a 30 day period is an indication of the severity of root rot disease. These counts are compared with the number of leaves lost per plant on uninoculated plants during the same period (Table 23). The data converted into % of defoliation are shown in Table 24.

It seems that healthy papaya plants characteristically drop their lower leaves through senescence during the 30 days period between the 1st and 2nd month of growth after germination. Most papaya lines dropped between 3 and 5 leaves which is between 20 and 30 percent of their leaves within a 1-month period (Tables 23 and 24). An exception was noted in Line 8 and 'Kapoho' Solo, which dropped about one leaf per plant which is only about 6 percent of the leaves. Only these lines were significantly different from the other uninoculated lines. These same two lines dropped 6 leaves per plant when inoculated with P. palmivora, which is about 45%. The rest of the inoculated lines dropped between 7 and 10 leaves per plant which is from 41% to 70%. For inoculated lines, the most severely affected was the susceptible line 'Higgins' while Line 8 and 'Kapoho' were the least affected.

In comparing inoculated with uninoculated papaya lines, differences in number and percent defoliation (Tables 23 and 24) were highly significant for most lines, with the exception of 'Waimanalo'-24 (Table 23) and the cross between the two sublines of 'Waimanalo'.

Most lines were severely defoliated regardless of whether or not they appeared to have some resistance.

Retardation of growth or reduced vigor of papaya plants due to root

TABLE 23. -- Comparison of number of leaves lost per plant from inoculated and uninoculated papaya lines during one month's growth

Lines and Hybrids	Greenhouse Inoculated 10 reps.	Outdoors Uninoculated 3 reps.	Difference	$S_{\bar{d}}$	t	t'
Waimanalo-23 x Waimanalo-24	6.4	3.5	2.9	1.81	1.60 ns	
Waimanalo-24	6.7	3.3	3.4	1.69	2.01 ns	
40	8.2 ± 1.9	4.5 ± 0.2	3.7			6.27 **
40 x Waimanalo-23	8.1	4.3	3.8	0.94	4.04 **	
40 x Waimanalo-24	8.0	4.2	3.8	1.52	2.50 *	
Waimanalo-23	7.6 ± 3.0	3.6 ± 0.3	4.0			4.44 **
Waimanalo-24 x Higgins	8.1	3.9	4.2	1.23	3.41 **	
40 X 45-T_{22}	7.5 ± 1.7	3.1 ± 0.2	4.4			8.46 **
40 x Higgins	9.0	4.4	4.6	0.96	4.79 **	
Waimanalo-23 x Higgins	8.8	4.2	4.6	1.15	4.00 **	
45-T_{22}	7.5	2.8	4.7	1.58	2.97 *	
Waimanalo-23 x 45-T_{22}	8.3	3.4	4.9	0.98	5.00 **	
45-T_{22} x Higgins	8.8	3.8	5.0	1.39	3.60 **	
45-T_{22} x Waimanalo-24	8.6	3.5	5.1	1.05	4.86 **	
Kapoho Solo	6.2	0.8	5.4	0.91	5.93 **	
Line 8	6.4	0.8	5.6	1.00	5.60 **	
Higgins	10.0	3.5	6.5	0.94	6.91 **	
Means	7.9	3.4				
K-ratio t (0.05)	1.3	1.2				

TABLE 24. -- Comparison of percent defoliation of inoculated and uninoculated papaya lines during one month's growth

Lines and Hybrids	Greenhouse Inoculated 10 reps. %	Outdoors Uninoculated 3 reps. %	Difference	$S_{\bar{d}}$	t	t'
Waimanalo-23 x Waimanalo-24	41.4	23.1	18.3	10.24	1.79 ns	
Waimanalo-24	44.2	21.8	22.4	10.11	2.22 *	
Waimanalo-23	47.9 ± 17.8	23.7 ± 1.2	24.2			4.47 **
40 x Waimanalo-23	53.9	28.4	25.5	5.37	4.75 **	
40	55.4	29.6	25.8	6.36	4.06 **	
40 x Waimanalo-24	53.3	27.1	26.2	8.82	2.97 *	
Waimanalo-24 x Higgins	56.8	27.0	29.8	7.75	3.85 **	
Waimanalo-23 x Higgins	58.9	28.4	30.5	6.50	4.69 **	
40 x 45-T_{22}	53.2 ± 10.6	22.2 ± 0.2	31.0			9.66 **
40 x Higgins	61.1	29.4	31.7	5.72	5.54 **	
Waimanalo-23 x 45-T_{22}	57.6	22.7	34.9	5.56	6.28 **	
45-T_{22}	56.0	19.6	36.4	9.68	3.76 **	
45-T_{22} x Waimanalo-24	59.8	22.6	37.2	6.89	5.40 **	
45-T_{22} x Higgins	62.6	25.3	37.3	7.54	4.95 **	
Line 8	45.9 ± 12.2	6.4 ± 1.9	39.5			10.34 **
Kapoho Solo	49.4	6.0	43.4	6.08	7.14 **	
Higgins	70.4	24.0	46.4	4.90	9.47 **	
Means	54.6	22.8				
K-ratio t (0.05)	8.0	7.2				
(0.01)	10.3	9.3				

rot has been mentioned as an important disease symptom (Parris, 1942; Trujillo and Hine, 1964). However, no report has mentioned to what extent vigor is reduced. Because of its relevance in regard to the present discussion, data on plant height 1 month after germination and before inoculation of lines grown in the greenhouse and outdoors were taken and are listed in Table 25. Comparison of plant height in lines grown in the greenhouse measured 1 month after inoculation and uninoculated controls grown outdoors was made (Table 26). To further study the effects of the disease on plant height, a comparison of increase in plant height during the 1-month inoculation period, between both sets of lines was also made (Table 27).

The results in Table 25 show that differences in plant height measured 30 days after germination between papaya lines grown in the greenhouse are significant only at 5% level of probability. The same lines growing outdoors showed no real differences. In comparing both sets of lines the mean height of all plants grown in the greenhouse was greater than that of those grown outdoors. Statistical differences were, however, detected only in about half the number of lines tested. These differences in growth may be explained by the higher temperatures and slightly reduced sunlight in the greenhouse compared to conditions occurring outdoors, which favor faster growth in papaya plants (Lange, 1961).

After inoculation, inoculated lines growing in the greenhouse had plants shorter than the same lines not inoculated growing outdoors. In spite of the more favorable growing temperatures in the greenhouse, some lines were severely affected by the disease.

The most affected line was the highly susceptible 'Higgins', and

TABLE 25. -- Comparison of plant height before inoculation, between papaya lines growing in a greenhouse and outdoors

Lines and Hybrids	Greenhouse 10 reps. cm	Outdoors 5 reps. cm	Difference cm	$S_{\bar{d}}$ cm	t	t'
40 x 45-T_{22}	4.0 ± 1.5	2.9 ± 0.3	1.1			2.21 ns
Waimanalo-23 x 45-T_{22}	3.8 ± 1.3	2.8 ± 0.4	1.0			2.09 ns
45-T_{22} x Higgins	3.8	2.8	1.0	0.48	1.98 ns	
40 x Waimanalo-23	3.7 ± 0.9	2.8 ± 0.1	0.4			1.58 ns
40 x Higgins	3.6	2.6	1.0	0.59	1.66 ns	
Waimanalo-23	3.5 ± 0.7	2.6 ± 0.1				3.91 **
45-T_{22}	3.5	2.7	0.9	0.36	2.44 *	
40 x Waimanalo-24	3.5 ± 1.2	2.6 ± 0.2	0.9			2.52 *
45-T_{22} x Waimanalo-24	3.5	2.7	0.8	0.36	2.08 ns	
Waimanalo-24 x Higgins	3.4 ± 1.2	2.4 ± 0.3	1.0			2.50 *
Kapoho Solo	3.3	2.6	0.6	0.14	4.43 **	
40	3.2	2.8	0.4	0.36	1.08 ns	
Waimanalo-24	3.2	2.7	0.5	0.22	2.27 *	
Higgins	3.2	2.3	0.9	0.24	3.71 **	
Waimanalo-23 x Higgins	3.2	2.4	0.8	0.26	3.12 **	
Waimanalo-23 x Waimanalo-24	2.9	2.3	0.5	0.32	1.59 ns	
Line 8	2.8	2.2	0.5	0.35	1.54 ns	
Means	3.4	2.6				
K-ratio t (0.05)	0.9	ns				

TABLE 26. -- Comparison of plant height of inoculated and uninoculated papaya lines one month after inoculation

Lines and Hybrids	Outdoors Uninoculated 3 reps. cm	Greenhouse Inoculated 10 reps cm	Difference cm	$S_{\bar{d}}$ cm	t	Growth reduct. % of control
Line 8	7.3	7.9	-0.6	0.90	0.66 ns	-8.2
Waimanalo-23	10.5	9.9	0.6	1.89	0.33 ns	5.7
40 x Waimanalo-23	10.9	9.7	1.2	2.00	0.62 ns	11.0
40 x 45-T_{22}	12.1	9.9	2.2	2.10	1.06 ns	18.2
Kapoho Solo	8.5	6.9	1.6	0.71	2.25 *	18.8
Waimanalo-23 x 45-T_{22}	12.8	9.8	2.9	2.20	1.33 ns	22.7
Waimanalo-23 x Waimanalo-24	11.8	9.1	2.7	2.50	1.07 ns	22.9
Waimanalo-24 x Higgins	10.8	8.3	2.5	1.60	1.52 ns	23.2
40	10.8	7.9	2.9	1.62	1.79 ns	26.9
45-T_{22} x Waimanalo-24	12.8	9.2	3.6	1.90	1.97 ns	28.1
Waimanalo-24	12.2	8.7	3.5	2.01	1.76 ns	28.7
Waimanalo-23 x Higgins	12.0	8.5	3.5	1.30	2.71 *	29.2
45-T_{22}	13.7	9.5	4.2	1.91	2.17 ns	30.7
40 x Waimanalo-24	12.5	8.3	4.2	1.40	3.05 *	33.6
45-T_{22} x Higgins	14.4	9.4	5.0	1.60	3.12 **	34.7
Higgins	10.7	6.9	3.8	0.66	5.77 **	35.5
40 x Higgins	13.4	8.6	4.9	1.60	3.01 *	36.6
Means	11.6	8.7				
K-ratio t (0.05)	3.2	2.0				
(0.01)	4.1	2.6				

TABLE 27. -- Comparison of increase in plant height of inoculated and uninoculated papaya lines during a 30-day period between the 1st and 2nd month after germination

Lines and Hybrids	Outdoors Uninoculated 3 reps. cm/month	Greenhouse Inoculated 10 reps. cm/month	Difference cm/month	$S_{\bar{d}}$ cm/month	t
Line 8	5.0	5.1	-0.1	0.96	0.10 ns
Waimanalo-23	7.9	6.3	1.6	1.74	0.92 ns
Kapoho Solo	6.0	4.0	2.0	1.05	1.90 ns
40 x Waimanalo-23	8.1	6.0	2.1	1.66	1.27 ns
40	7.8	4.8	3.0	1.31	2.29 *
Waimanalo-23 x Waimanalo-24	9.3	6.3	3.0	2.21	1.36 ns
40 x 45-T_{22}	9.2	6.0	3.2	1.78	1.80 ns
Waimanalo-24 x Higgins	8.3	5.0	3.3	1.13	2.92 *
Waimanalo-23 x 45-T_{22}	9.9	6.1	3.8	1.76	2.16 ns
Waimanalo-24	9.5	5.5	4.0	1.98	2.02 ns
Waimanalo-23 x Higgins	9.3	5.2	4.1	1.20	3.42 **
45-T_{22} x Waimanalo-24	9.9	5.7	4.2	1.46	2.88 *
Higgins	8.1	3.7	4.4	0.56	7.86 **
45-T_{22}	10.8	6.0	4.8	1.56	3.08 *
40 x Waimanalo-24	9.8	4.8	5.0	0.97	5.15 **
40 x Higgins	10.5	5.0	5.5	1.30	4.23 **
45-T_{22} x Higgins	11.3	5.7	5.6	1.33	4.21 **
Means	8.9	5.3			
K-ratio t (0.05)	1.6	3.1			
(0.01)	2.1	4.0			

the hybrid between the moderately resistant 45-T_{22} and 'Higgins'. It was surprising to find that 45-T_{22} was not significantly affected, whereas 'Kapoho' another line with the same level of resistance, was affected severely. Inoculated resistant lines showed no significant difference from their uninoculated counterparts even though the plants were slightly shorter.

Taking the increase in plant height during the 30 day period of growth in the nursery into consideration only the most resistant lines, Line 8, 'Waimanalo'-23 and 24, were not significantly different from their uninoculated counterparts. The same was true for most hybrids having those lines as parents. Exceptions were found in cases where either 'Higgins' or 45-T_{22} or even when line 40 acted as the other parent. Since the growth rate of most lines was affected by the disease, the lack of significance in plant height (Table 26) for lines 40, 45-T_{22}, and certain hybrids may have been due to better environment for growth in the greenhouse.

It has been demonstrated previously that most inoculated lines with the exception of line 45-T_{22} and the crosses 45-T_{22} x 'Waimanalo'-24 and 45-T_{22} x 'Higgins' (Table 22), did not show a decrease in growth rate as measured by number of nodes compared to controls. Increase in plant height and plant height itself at the end of the inoculation period were, however, affected in many lines. This is probably an indication that cell elongation is affected but cell division is not. This resulted in reduction in height of the lines more severely affected by the disease. This is probably due to the inability of severely affected plants to absorb adequate nutrients to support optimum elongation of the plant. Resistant lines are able to

produce new roots faster than moderately resistant or susceptible lines, and this has been demonstrated in other species attacked by Phytophthora (Newhook, 1961). In this study, new root production was observed in several lines about 2 weeks after inoculation.

Kanlong and Hendrix (1977) found that a single Phytophthora isolate could have the ability to kill plants or the ability to merely stunt growth when plants of different species were planted into inoculated soil. This study tested only one isolate of P. palmivora (P170), which was able to kill plants of all papaya lines tested. Several lines showed low mortality however, and were therefore considered resistant. This isolate was also able to reduce internode length, in susceptible and moderately resistant lines, but not in resistant lines. Regardless of level of resistance, the isolate was able to severely defoliate the plants. Thus, in this case, isolate P170 produced different reaction of the hosts that seems to be dependent on the level of resistance of the particular papaya line attacked.

To further assess the effect of root rot on growth of papaya plants, stem diameter was also measured on the same dates as plant height both in the greenhouse and outdoors (Table 28). Stem diameter measurements were also taken one month after inoculation, in both inoculated and uninoculated lines and the increases in stem diameter in both sets of lines are presented in Tables 29 and 30.

Stem diameter of papaya plants measured 1 month after germination does not appear to be affected by higher temperature contrary to the response obtained with plant height. It may also mean that at this juvenile stage plants are still in the primary growth stage, and secondary growth has not yet been initiated.

TABLE 28. -- Comparison of stem diameter of papaya lines grown in a green house and outdoors before inoculation

Lines and Hybrids	Greenhouse 10 reps. mm	Outdoors 3 reps. mm	Difference mm	$S_{\bar{d}}$ mm	t	t'
40 x Higgins	3.0	2.7	0.3	0.45	0.78 ns	
40	2.9	2.8	0.1	0.48	0.29 ns	
Higgins	2.9	2.6	0.3	0.37	0.86 ns	
40 x Waimanalo-24	2.9	2.5	0.4	0.45	0.84 ns	
Waimanalo-23 x Higgins	2.9	2.5	0.4	0.36	1.14 ns	
45-T_{22} x Higgins	2.9	2.6	0.3	0.45	0.69 ns	
Waimanalo-24 x Higgins	2.9	2.4	0.4	0.41	1.07 ns	
Waimanalo-24	2.8	2.5	0.3	0.35	0.83 ns	
40 x Waimanalo-23	2.8	2.6	0.2	0.40	0.58 ns	
40 x 45-T_{22}	2.8	2.3	0.5	0.32	1.56 ns	
Waimanalo-23	2.7	2.3	0.4	0.28	1.43 ns	
Waimanalo-23 x 45-T_{22}	2.6	2.3	0.3	0.40	0.75 ns	
45-T_{22}	2.5	2.1	0.4	0.41	0.95 ns	
45-T_{22} x Waimanalo-24	2.5	2.4	0.1	0.36	0.39 ns	
Waimanalo-23 x Waimanalo-24	2.4	2.3	0.1	0.39	0.23 ns	
Kapoho Solo	2.2 $\pm$ 0.09	2.0 $\pm$ 0.3	0.2			1.93 ns
Line 8	2.1	1.9	0.2	0.14	1.57 ns	
Means	2.7	2.4				
K-ratio t (0.05)	0.3	0.3				
(0.01)	0.4	0.4				

TABLE 29. -- Comparison of stem diameter of inoculated and uninoculated papaya lines one month after inoculation

Lines and Hybrids	Outdoors Uninoculated 3 reps. mm	Greenhouse Inoculated 10 reps. mm	Difference mm	$S_{\bar{d}}$ mm	t
Line	4.6	4.3	0.3	0.30	1.00 ns
Kapoho Solo	4.4	4.0	0.4	0.36	1.11 ns
Waimanalo-23	6.1	5.0	1.1	0.56	2.00 ns
40 x 45-T_{22}	6.1	4.8	1.3	0.55	2.45 *
Waimanalo-23 x Higgins	6.5	5.0	1.5	0.42	3.67 **
Waimanalo-23 x Waimanalo-24	6.3	4.8	1.6	0.69	2.28 *
40 x Waimanalo-23	6.7	5.0	1.7	0.66	2.59 *
Higgins	6.7	4.9	1.8	0.26	6.96 **
40 x Waimanalo-24	6.8	5.0	1.8	0.49	3.76 **
Waimanalo-24	6.8	4.8	2.0	0.57	3.54 **
Waimanalo-24 x Higgins	7.0	5.0	2.0	0.62	3.24 **
40 x Higgins	7.3	5.2	2.1	0.51	4.06 **
Waimanalo-23 x 45-T_{22}	6.7	4.6	2.1	0.62	3.40 **
45-T_{22}	6.5	4.3	2.2	0.57	3.89 **
45-T_{22} x Waimanalo-24	6.8	4.6	2.2	0.53	4.09 **
40	7.2	4.8	2.4	0.69	3.39 **
45-T_{22} x Higgins	7.3	4.8	2.5	0.50	5.00 **
Means	6.5	4.8			
K-ratio t (0.05)	0.6	0.6			
(0.01)	0.7	ns			

TABLE 30. -- Comparison of increase in stem diameter of inoculated and uninoculated papaya lines during a 30-day period between the 1st and 2nd month after germination

Lines and Hybrids	Outdoors Uninoculated 3 reps. mm/month	Greenhouse Inoculated 10 reps. mm/month	Difference mm/month	$S_{\bar{d}}$ mm/month	t	t'
Line 8	2.6	2.3	0.3	0.32	0.94 ns	
Kapoho Solo	2.3	1.7	0.6	0.36	1.67 ns	
Waimanalo-23	3.4	2.3	1.1	0.45	2.44 *	
Waimanalo-23 x Waimanalo-24	3.5	2.3	1.2	0.36	3.33 **	
Waimanalo-23 x Higgins	3.4 $\pm$ 0.05	2.2 $\pm$ 0.5	1.2			7.44 **
40 x Waimanalo-23	3.5	2.2	1.3	0.41	3.17 **	
40 x 45-T_{22}	3.3	2.0	1.3	0.39	3.33 **	
Higgins	3.5	2.0	1.5	0.28	5.36 **	
Waimanalo-24	3.7	2.1	1.6	0.45	3.56 **	
40 x Waimanalo-24	3.7	2.1	1.6	0.24	6.67 **	
45-T_{22} x Waimanalo-24	3.7	2.1	1.6	0.36	4.44 **	
40 x Higgins	3.9	2.2	1.7	0.26	6.54 **	
40	3.8	2.0	1.8	0.33	5.45 **	
Waimanalo-23 x 45-T_{22}	3.8	2.0	1.8	0.37	4.86 **	
Waimanalo-24 x Higgins	3.9	2.1	1.8	0.36	5.00 **	
45-T_{22} x Higgins	4.0	2.0	2.0	0.42	4.76 **	
45-T_{22}	3.9	1.8	2.1	0.30	7.00 **	
Means	3.5	2.1				
K-ratio t (0.05)	0.4	ns				
(0.01)	0.6					

One month later, after one set of papaya lines had been inoculated, most of the inoculated lines showed lower stem diameter than the uninoculated controls (Table 29). Line 8, 'Waimanalo'-23 and 'Kapoho' Solo however failed to show this effect. This also happened with increase in stem diameter during the 30-day period (Table 30) except that for this variable, 'Waimanalo'-23 was affected. Differences detected in stem diameter within inoculated lines were at the 5% level (Table 29). In the case of increase in stem diameter no significant differences were detected within inoculated lines (Table 30). The reason that 'Kapoho' showed a response similar to that of resistant lines is not clear but it may be that it grows at slower rate than other lines. Line 8 which is also a slow grower has been shown to be a resistant line. It did not appear to be greatly affected in stem diameter by the disease. 'Waimanalo'-23, another resistant line was slower in increase in stem diameter compared to the control, but stem diameter measured 1 month after inoculation failed to show any difference compared to the control. This suggests that survivors were able to overcome the effects of the disease. Increase in stem diameter appears to be distinctly affected by root rot in resistant lines like 'Waimanalo'-24 and Line 40 as well as their F_1 hybrids.

FOLLOW UP AFTER TRANSPLANTING

Survivors of each line from greenhouse inoculation were selected at random, and planted in the field approximately one month after inoculation along with uninoculated seedlings of the same lines.

Data on percent mortality recorded up to 3 months after transplanting are shown in Table 31. No plant in any of the lines died after

TABLE 31. -- Mean percent mortality of inoculated and uninoculated papaya lines recorded 3 months after transplanting in infested field

Lines and Hybrids	Inoculated			Uninoculated			Mean lines	
	Retransf. %	Transf. angle		Transf. angle	Retransf. %		Transf. angle	Retransf. %
40 x Waimanalo-24	1.1	6.12	=	0.81	0		3.47	0.4
Waimanalo-23 x Waimanalo-24	3.3	10.54	=	0.81	0		5.68	1.0
Waimanalo-24	4.5	12.29	=	0.81	0		6.55	1.3
40	5.8	13.89	=	0.81	0		7.35	1.6
40 x Waimanalo-23	5.8	13.89	=	0.81	0		7.35	1.6
Line 8	2.2	8.58	=	8.58	2.2		8.58	2.2
Waimanalo-23 x 45-T_{22}	8.3	16.74	=	0.81	0		8.78	2.3
Waimanalo-23 x Higgins	8.3	16.74	=	0.81	0		8.78	2.3
45-T_{22} x Waimanalo-24	9.1	17.60	>	0.81	0		9.20	2.6
Waimanalo-24 x Higgins	2.2	8.58	=	13.89	5.8		11.24	3.8
40 x 45-T_{22}	16.0	23.62	>	0.81	0		12.22	4.5
Kapoho Solo	5.8	13.89	=	16.35	7.9		15.12	6.8
Waimanalo-23	18.4	25.37	>	6.12	1.1		15.74	7.4
40 x Higgins	40.1	39.29	>	0.81	0		20.05	11.8
45-T_{22}	37.1	37.54	=	21.72	13.7		29.63	24.4
45-T_{22} x Higgins	41.3	40.00	>	19.26	10.9		29.63	24.4
Higgins	94.6	76.54	>	16.74	8.3		46.64	52.9
Mean disease conditions	14.5	22.42	>	6.51	1.3		14.47	6.2

Comparisons in the transformed scale between:
Two line or hybrid means: K-ratio t (0.01) = 15.65
Two means for disease condition: K-ratio t (0.01) = 4.01
Two means of disease condition for the same line: K-ratio t (0.01) = 16.51
Two lines at same dis. cond. or different: LSD (0.01) = 22.65

the third month. Means of disease ratings are presented in Table 32.

In general, percent mortality was higher among inoculated lines than among uninoculated lines although significant differences were not detected in some cases. Results generally demonstrate that mortality in inoculated plants was higher because of a carry over effect from artificial inoculation performed at one month of age in the greenhouse. The carry over effect was present in spite of the Banrot 40W fungicide drench applied to all survivors before transplanting one month after inoculation. The low effectiveness of the fungicide as a therapeutant was recognized by the fact that P. palmivora was reisolated 1 week after drenching before transplanting and from old lesions in roots of stunted plants in the field as long as 4 months after transplanting.

The same gradation in the order of resistance observed in the greenhouse (Table 10) was observed in the field for inoculated plants. The same tendency was also observed in uninoculated plants after transplanting into the field, even though significant differences were not detected. It was noted that 45-T_{22}, 'Higgins', 'Kapoho', and the hybrid 45-T_{22} x 'Higgins' showed the highest mortality among the uninoculated plants. The lack of detection of differences, however, confirms the observation of Nakasone and Aragaki (1973) of the unpredictable distribution and concentration of the pathogen in the field. The pathogen was present in the field in this planting, as shown by isolation from dead and stunted plants (Fig. 67), which were healthy and uninoculated when transplanted into the field. Other species of fungi such as Pythium and Rhizoctonia were also isolated from some of these plants. Environmental conditions were not considered ideal for optimum development of the disease in the field because of

TABLE 32. -- Mean disease ratings of inoculated and uninoculated papaya lines recorded 4 months after transplanting in infested field

Lines and Hybrids	Inoculated		Uninoculated	Mean lines and Hybrids
40	3.7	<	4.7	4.2
40 x Waimanalo-24	3.8	=	4.5	4.2
Waimanalo-23 x Waimanalo-24	3.7	=	4.4	4.1
Waimanalo-23 x 45-T_{22}	3.4	<	4.8	4.1
Line 8	4.1	=	4.0	4.0
Waimanalo-24	3.5	<	4.5	4.0
40 x Waimanalo-23	3.5	=	4.4	4.0
Waimanalo-23 x Higgins	3.6	=	4.4	4.0
40 x 45-T_{22}	3.3	<	4.4	3.9
45-T_{22} x Waimanalo-24	3.4	=	4.2	3.8
Kapoho Solo	3.8	=	3.5	3.7
Waimanalo-23	3.1	<	4.1	3.6
Waimanalo-24 x Higgins	3.2	=	4.1	3.6
40 x Higgins	2.6	<	4.4	3.5
45-T_{22}	2.4	<	3.4	2.9
45-T_{22} x Higgins	2.2	=	3.0	2.6
Higgins	1.1	<	3.4	2.3
Mean disease condition	3.2	<	4.1	3.7

Comparison between:
Two line or hybrid means: K-ratio t (0.01) = 0.9
Two means for disease condition: K-ratio t (0.01) = 0.2
Two means of disease cond. for same line: K-ratio t (0.01) = 0.9
Two lines at same dis. cond. or different: LSD (0.01) = 1.1

delayed rainy season during 1976.

In the analysis of variance of the disease ratings resistant lines 'Waimanalo'-23 and 24, as well as line 40 and some of their hybrids showed lower vigor in inoculated plants than in uninoculated ones. This provided evidence that in spite the fungicide drench, the pathogen was still present in the roots at transplanting time and thereafter. The possibility of new infections in the field was not precluded. The similarity of tendencies to discriminate the lines by their resistance by the use of both scales (% mortality and rating scale) can be shown by the correlation between them calculated using data from Tables 31 and 32: (1) r = - 0.9583 ** for previously inoculated plants; (2) r = - 0.9096 ** for uninoculated plants; and (3) r = - 0.9359 ** for the mean of both sets. The advantage of using the disease rating scale over percent mortality is shown by the lower coefficient of variation obtained with the former: CV = 62.6% for % mortality transformed to angles; CV = 13.9% for disease rating scale.

The greater vigor shown by uninoculated lines suggests that even when the pathogen was present in the field, it did not have favorable environmental conditions for development. The fact that the organism was present in the field is shown by the marked reduction in vigor of the uninoculated susceptible and moderately resistant lines compared to the resistant lines i.e. 'Higgins' vs. 40 or 45-T_{22} x 'Higgins' vs. 'Waimanalo'-24 (Table 32).

Data on mean plant height and mean stem diameter recorded 1 month, 3 months and 4 months after transplanting of lines inoculated one month after germination and uninoculated check plants are shown in Tables 33 and 34. Data show that one month after transplant all inoculated lines

TABLE 33. -- Mean plant height of inoculated and uninoculated papaya lines 1, 3 and 4 months after transplanting in infested field

Lines and Hybrids	1 month			3 months			4 months		
	Inoc.	Uninoc.	Line mean	Inoc.	Uninoc.	Line mean	Inoc.	Uninoc.	Line mean
	cm	cm	cm	cm	cm	cm	cm	cm	cm
Line 8	33.1 =	39.6	36.4	96.1 =	102.4	99.2	124.7 =	128.0	126.3
Kapoho Solo	34.2 <	42.7	38.5	92.7 =	98.4	95.6	121.1 =	118.1	119.6
Waimanalo-23 x Higgins	32.9 <	42.9	37.9	89.7 =	103.1	96.4	113.9 =	127.0	120.4
45-T_{22} x Waimanalo-24	31.8 <	47.3	39.5	87.6 =	109.4	98.5	111.5 =	128.7	120.1
Waimanalo-23 x Waimanalo-24	31.1 <	43.6	37.4	85.7 =	102.5	94.1	109.0 =	121.5	115.2
Waimanalo-24	33.3 <	44.4	38.9	83.4 =	101.4	92.4	107.2 =	123.7	115.5
40 x Waimanalo-23	27.1 <	38.0	32.1	82.2 =	97.3	89.8	109.7 =	121.0	115.3
Waimanalo-24 x Higgins	28.1 <	42.7	35.4	80.5 =	101.4	91.0	105.5 =	125.2	115.4
45-T_{22} x Higgins	28.4 <	45.0	36.7	78.1 =	85.6	81.9	93.4 =	103.3	98.3
Waimanalo-23 x 45-T_{22}	33.0 <	51.7	42.4	92.2 <	117.8	105.0	118.9 =	137.4	128.2
40 x Higgins	29.0 <	48.1	38.6	82.6 <	110.9	96.8	118.0 =	132.1	125.0
Waimanalo-23	27.0 <	43.2	35.1	81.6 <	106.8	94.2	108.2 =	126.3	117.2
40	30.3 <	46.6	38.5	90.7 <	111.7	101.2	116.3 <	140.4	128.3
40 x 45-T_{22}	31.1 <	51.2	41.2	88.2 <	116.9	102.6	111.4 <	140.5	125.9
40 x Waimanalo-24	26.9 <	46.6	36.7	83.2 <	114.0	98.6	109.9 <	132.7	121.3
45-T_{22}	30.2 <	45.0	37.6	69.3 <	94.3	81.8	88.3 <	115.6	102.0
Higgins	20.9 <	39.3	30.1	35.8 <	82.8	59.3	45.0 <	105.6	75.3
Mean disease condition	29.9 <	44.6	37.2	82.3 <	103.3	92.8	106.6 <	125.1	115.9
K-ratio t (0.05)			ns			18.2			18.5
(0.01)			ns			23.6			24.1

TABLE 34. -- Mean stem diameter per plant of inoculated and uninoculated papaya lines 1, 3 and 4 months after transplanting in infested field

Lines and Hybrids	1 month			3 months			4 months		
	Inoc. mm		Uninoc. mm	Line mm	Inoc. mm		Uninoc. mm	Line mm	Inoc. mm		Uninoc. mm	Line mm
Line 8	15.6	=	18.3	17.0	45.2	=	45.8	45.5	69.4	=	63.9	66.7
Kapoho Solo	16.8	<	20.7	18.8	46.1	=	47.1	46.6	66.8	=	64.1	65.5
Waimanalo-23 x Higgins	15.4	<	21.6	18.5	44.1	=	49.3	46.7	66.9	=	69.9	68.4
45-T_{22} x Higgins	14.7	<	22.3	18.5	43.7	=	43.6	43.7	63.3	=	61.6	62.5
40 x Waimanalo-23	12.9	<	18.0	15.5	38.7	=	45.0	41.9	59.6	=	65.1	62.4
Waimanalo-23 x 45-T_{22}	15.8	<	25.4	20.6	46.0	<	54.7	50.4	68.8	=	73.2	71.0
40 x Higgins	14.8	<	24.3	19.5	41.8	<	53.2	47.5	67.8	=	72.3	70.1
45-T_{22} x Waimanalo-24	15.8	<	23.2	19.5	43.5	<	52.8	48.2	64.4	=	70.8	67.6
40 x Waimanalo-24	13.5	<	21.8	17.6	39.2	<	50.9	45.0	64.0	=	71.9	67.9
Waimanalo-23	12.5	<	20.9	16.7	39.1	<	49.4	44.2	63.9	=	70.0	66.9
40 x 45-T_{22}	14.0	<	24.6	19.3	42.6	<	53.4	48.0	63.1	=	72.3	67.7
Waimanalo-24 x Higgins	15.1	<	22.6	18.8	40.1	<	49.9	45.0	62.2	=	71.5	66.9
Waimanalo-24	15.5	<	22.4	19.0	39.6	<	49.4	44.5	61.5	=	70.8	66.2
40	14.6	<	20.8	17.7	40.0	<	49.0	44.5	59.9	=	71.7	65.8
Waimanalo-23 x Waimanalo-24	14.9	<	20.4	17.6	39.3	<	47.2	43.2	59.6	=	65.9	62.7
45-T_{22}	14.3	<	21.5	17.9	37.6	<	48.5	43.1	55.3	=	66.8	61.1
Higgins	12.9	<	22.2	17.6	22.9	<	45.7	34.3	27.3	<	68.2	47.7
Mean disease condition	14.6	<	21.8	18.2	40.6	<	49.1	44.8	61.4	<	68.8	65.1
K-ratio t (0.05)				ns				10.5				9.6
(0.01)				ns				ns				12.4

and hybrids, with the exception of Line 8, were significantly shorter and with thinner stems than their uninoculated counterparts. This demonstrates that P. palmivora inoculum applied two months earlier in the greenhouse was still active in reducing growth of the surviving plants three months later.

When the plants were measured again at the end of the third month after transplanting, lines such as 'Waimanalo'-24, 'Kapoho', and some hybrids showed signs of recovery, inasmuch as their height was not significantly different from the controls. For stem diameter, 'Waimanalo'-24 was observed to have less stem diameter than controls.

By the fourth month after transplanting, most inoculated lines and hybrids had recovered in height and stem diameter. Exceptions regarding height were lines 40, 45-T_{22} and 'Higgins' as well as a few hybrids (Table 33). In stem diameter the only exception was the susceptible 'Higgins', which failed to recover (Table 34). Generally all inoculated lines were still shorter and thinner than uninoculated checks but no statistical differences were found.

Line 8 was the only one line which was not appreciably reduced in growth and showed among the lowest mortality from P. palmivora. Even though its survivors were severely defoliated compared to the control (Tables 23 and 24), it was the most resistant line of those tested. The two sublines of 'Waimanalo' Nos. 23 and 24, must also be considered somewhat resistant because of their low mortality, and their ability to recover from infection.

Both Line 8 and 'Waimanalo' were reported to show field tolerance by Nakasone and Aragaki (1973). This study provides further evidence that they are resistant as shown by the several experiments carried out.

Line 40 might also be considered resistant even though its vigor was slightly affected by the disease.

Figures 58 to 66 show inoculated and uninoculated 6-month old bearing trees in the replant field. This shows that survivors from inoculation are able to reach the reproductive stage, and yield progeny which would likely inherit the resistance to root rot since additive genetic variance was found to be considerably high.

PLATE VII

Inoculated survivors and uninoculated papaya lines and F_1 hybrids, shown 6 months after transplanting

Figure 58. 'Waimanalo'-23. First row, survivors are two plants on the right; uninoculated are two plants on the left

Figure 59. Line 8. First row, survivors are two plants on the right; uninoculated are two plants on the left

Figure 60. 'Waimanalo'-24. First row, uninoculated are three plants on the right; survivors are three plants on the left

Figure 61. Line 40. First row, survivor is one plant on the left; uninoculated three plants on the right

Figure 62. 'Kapoho' Solo. First row, uninoculated are three plants on the right; survivors are three plants on the left

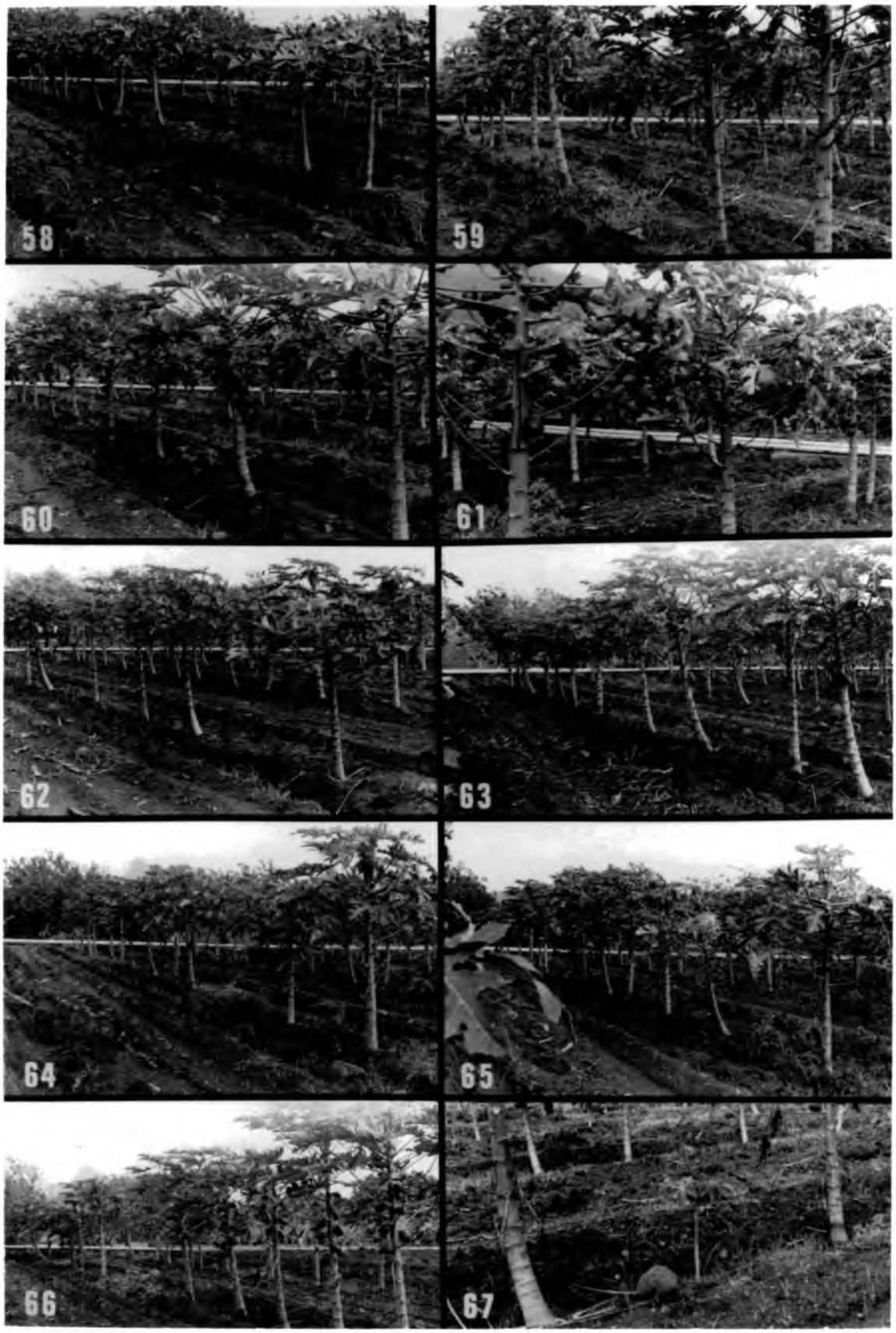
Figure 63. Line 45-T_{22}. First row, uninoculated are two plants on the right; survivors are two plants on the left

Figure 64. 'Waimanalo'-23 x 'Waimanalo'-24. First row, survivors are two plants on the right; uninoculated are two plants on the left

Figure 65. Line 40 x 'Waimanalo'-24. First row, survivors are two plants on the right; uninoculated are three plants on the left

Figure 66. 'Waimanalo'-23 x Line 45-T_{22}. First row, uninoculated are two plants on the right; survivors are three plants on the left

Figure 67. First row shows an uninoculated seedling of 'Higgins' stunted by root rot caused by P. palmivora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BACKCROSSING AND PHENOTYPIC RECURRENT SELECTION TO ACCUMULATE RESISTANCE TO ROOT ROT CAUSED BY *P. PALMIVORA* IN *CARICA PAPAYA*. A PROPOSAL

Although heritability estimates derived in this study come from information from selfed parents and F_1 progenies, and considering the fact only 5 parental lines were included, it is believed that high additive genetic variation for root rot resistance has been demonstrated. This can be used in formulating a breeding program to accumulate resistance in 'Kapoho' Solo, the current export papaya cultivar grown in Hawaii. The need for a resistant cultivar is urgent because of limited availability of virgin land needed to avoid replant problems due to root rot.

Further studies leading to the production of F_2's and backcrosses should also be undertaken to confirm the heritability estimates. Screening of the maximum number of papaya accessions from other areas should also be done to determine whether a better source of resistance could be found.

The proposal of a breeding method to accumulate resistance to root rot in 'Kapoho' Solo would rely on the screening procedure developed in this study to eliminate susceptible seedlings and maintaining only the most resistant survivors. Increasing the level of inoculum can be used to increase the selection intensity, thus maximizing the genetic advance. The transplanting of survivors into an infested field also provides a suitable environment to increase selection intensity, although less reliable than greenhouse inoculation.

Backcrossing is a breeding method which has been commonly used in both self-pollinated and cross-pollinated crops to incorporate disease

resistance into cultivars with highly desirable agronomic or horticultural characteristics (Allard, 1967). If the character to be incorporated has high heritability, as found in this study, backcrossing is a suitable method to use.

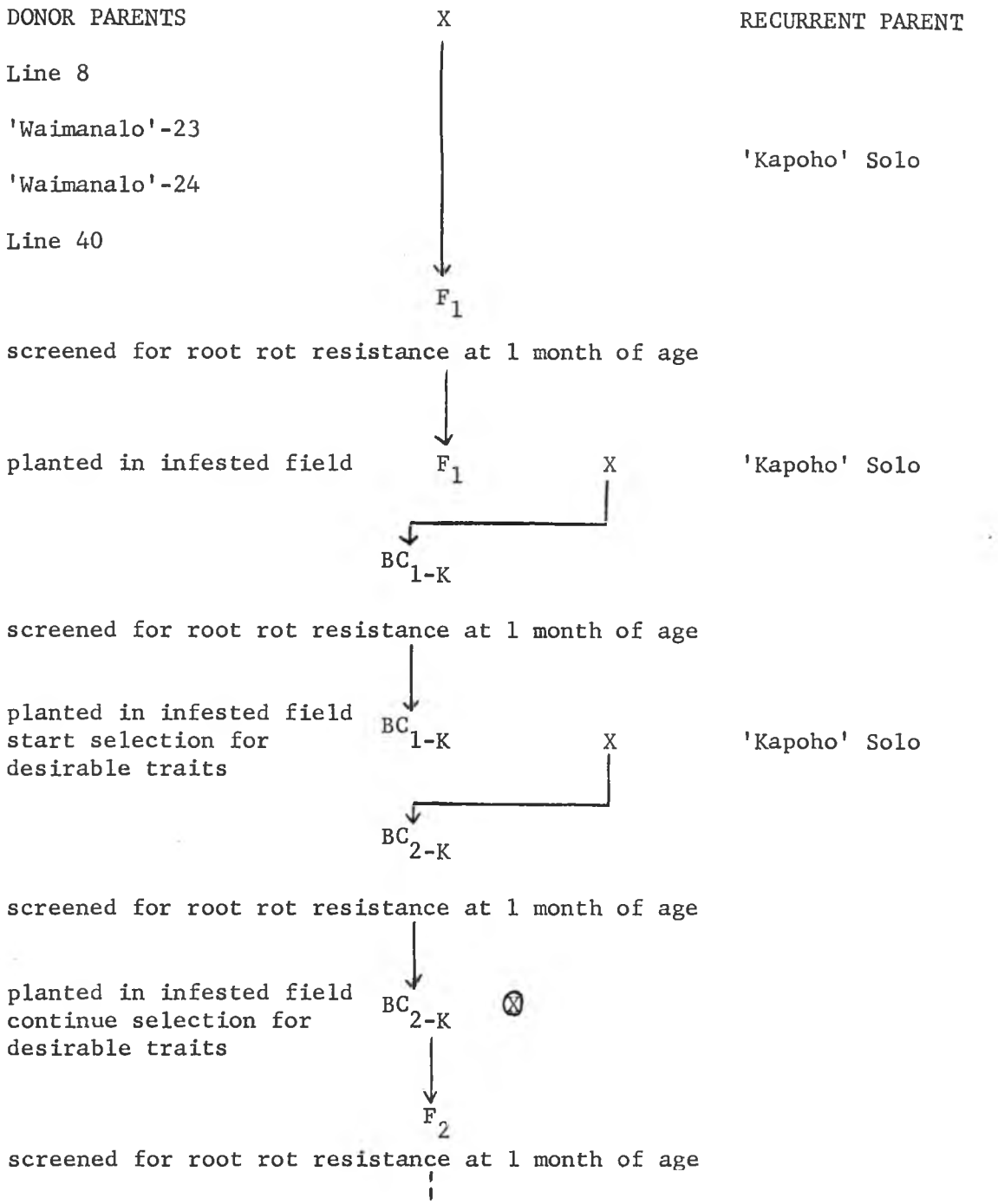
Penny et al. (1963) have indicated that phenotypic recurrent selection is effective in increasing gene frequency of desirable alleles, mainly when high additive genetic variation is available. If inbred papaya lines are the goal as appears to be the case in Hawaii, this method provides material with a large number of elite plants which can be inbred to achieve such a goal.

This proposal would include a breeding scheme combining a backcrossing program to initially incorporate the resistance from donor parents like Line 8, 'Waimanalo'-23 and/or 'Waimanalo'-24, and Line 40 into 'Kapoho' Solo. Selection would be done in each generation through use of the screening method already mentioned to insure maintenance of the most desirable plants. Phenotypic recurrent selection will also allow accumulation of resistance and maintenance of genetic variability in the population. Selfing of elite plants will provide superior inbred lines to develop resistant cultivars.

Other characters separated in the parents would have to be combined in such a breeding program (Nakasone, 1977 personal communication).

Donor parents	Advantages	Disadvantages
Line 8	Root rot resistance	Late bearing
	small fruit	soft flesh
'Waimanalo'	root rot resistance	
	early bearing	large fruit
Line 40	firm flesh	

Recurrent parent	Advantages	Disadvantages
'Kapoho' Solo	Wide adaptability small fruit firm flesh	Moderate root rot resistance Late bearing



F_2 Start recurrent selection program

Planted in infested field. Select for horticulturally desirable traits. Intercross selected plants within families.

Plant progenies from intercrossed families. Screen for root rot resistance at 1 month of age. Plant survivors in infested field. Select for horticulturally desirable traits. Self intercrossed families.

Plant progenies from selfed families. Screen for root rot resistance at 1 month of age. Plant in infested field. Select for horticulturally desirable traits. Intercross selected plants within families.

Plant intercrossed families. Screen for root rot resistance at 1 month of age. Plant survivors in infested field. Select for optimum combination of traits. Start selfing to derive inbred lines to be used as varieties.

SUMMARY AND CONCLUSIONS

One of the most important disease problems of papaya in Hawaii is root rot caused by Phytophthora palmivora Butl. This disease plays a major role in the 'replant problem". Field resistance has been detected but variable environmental conditions from year to year, as well as heterogeneous distribution and concentration of the pathogen in the field are obstacles to the rapid advance in breeding for resistance.

The objectives of the present research were: (1) to confirm the reliability of the existing method of screening for resistance in young papaya seedlings or to develop one; (2) to determine heritability of resistance to Phytophthora root rot in papaya using controlled inoculations; (3) to assess growth reduction caused by the disease; and (4) to follow up the growth performance of inoculated seedlings and survival of resistant papaya seedlings under field conditions.

A series of experiments was conducted using an existing laboratory-greenhouse inoculation procedure. This procedure makes use of small chambers where recently germinated papaya seedlings are suspended by the cotyledonary leaves while the roots are dipped into a zoospore suspension for a short time. Seedlings are later transplanted in pots and kept in the greenhouse for observation. This procedure was found not to be reliable since it was not able to differentiate between susceptible and resistant papaya lines. These lines had shown differences in resistance through having them evaluated previously for five successive plantings in infested fields.

During this research a new method of inoculation of papaya

seedlings with P. palmivora was developed. Isolate P170 was used throughout this study. The procedure requires that papaya seedlings be grown in the greenhouse for 1 month in peat pots using vermiculite as growing media. Four to 6 day old cultures of the fungus grown in 10% V-8 juice agar are used to produce sporangia. Sporangia are germinated in deionized water to produce a zoospore suspension. The zoospore suspension adjusted to the desired concentration is poured directly into peat pots where papaya seedlings are growing. Mortality counts and disease ratings were made one month after inoculation using a scale from 1 for dead plants to 5 for vigorous plants.

This method of inoculation confirmed the results of previous workers that there is a definite relationship between age of the seedlings and susceptibility to root rot. Younger papaya seedlings have significantly higher mortality than older ones. Very low levels of inoculum are enough to cause significant mortality in younger seedlings, whereas older seedlings require higher concentrations of inoculum to cause the same level of mortality. This was demonstrated using 'Higgins' the most susceptible papaya line found. No resistance was shown by 1 and 2-week old seedlings of any of three lines that had different level of resistance at older age. Somewhere after the second week after germination resistance begins to develop. Resistance is substantial in 1-month old and 2-month old seedlings. There was no difference between 2 and 3-month old seedlings. Mortality of papaya seedlings inoculated when 1-week old was 50% which is significant. Fifty percent mortality was obtained with a concentration as low as 200 sporangia/plant and 100% mortality was obtained with 5,000 sporangia/plant. With 1-month old seedlings 100% mortality was obtained with 9,000 sporangia/plant. A

concentration of 18,000 sporangia/plant was needed to obtain the same level of mortality with 2-month old seedlings. Since resistance is a relative term applied to different plant populations comparing their response to a disease, the resistance shown by the seedlings of one cultivar or line to a pathogen at different stages of growth has been called in this work developmental resistance. Increasing developmental resistance with older seedlings was shown in general by most papaya lines tested.

By testing increasing levels of inoculum on several 1 and 2-month old papaya lines, it was possible to demonstrate that some lines showed more resistance than others. From these experiments, 'Waimanalo'-23 and Line 40 were found resistant compared to 'Higgins'.

Using a uniform concentration of inoculum (5,250 sporangia/plant) determined from previous experiments, 17 papaya lines and F_1 hybrids were inoculated. Reliability of the inoculation procedure was supported by consistent greenhouse and field demonstrations. The resistance of papaya lines to root rot was classified experimentally as follows: resistant: Line 8, 'Waimanalo'-23, 'Waimanalo'-24 and Line 40; moderately resistant: 45-T_{22} and 'Kapoho' Solo; and susceptible: 'Higgins'. The correlation between percent mortality obtained in this test and percent mortality obtained in the same lines from several plantings in infested fields was 0.9355 **.

Heritability of resistance was estimated by analyzing transformed percent mortality and disease ratings of the progenies of a 5 x 5 half diallel. Two methods of statistical analysis were employed for both variables. When percent mortality was analyzed, Hayman's method gave narrow heritability of 42% and broad heritability of 57%. When disease

ratings were analyzed by the same method, a narrow heritability value of 47% and broad heritability value of 61% were obtained. Using Griffing's method 2 of analysis, narrow heritability for percent mortality was 89% and broad heritability was 91%. For disease ratings, narrow heritability was 91.6% and broad heritability was 91.9%. Considerably high additive genetic variance is present in the population, and dominance variance is negligible.

Since 'Kapoho' Solo the major commercial papaya cultivar on the Island of Hawaii has only a moderate level of resistance, a breeding program to incorporate genes for resistance from resistant papaya lines into it is proposed. A combination of backcrossing to incorporate the genes for resistance to root rot, followed by phenotypic recurrent selection to accumulate resistance, and subsequent derivation of inbred lines from elite plants could probably produce resistant varieties with desirable horticultural characteristics.

Defoliation and growth reduction caused by the disease were evaluated in inoculated seedlings at the nursery stage, by comparing them with uninoculated papaya seedlings of the same line or hybrid. Line 8 was shown to be the most resistant of all lines tested, since it was not adversely affected in growth by the disease although a few seedlings died and plants showed defoliation compared with uninoculated controls. Other resistant lines with low mortality were Line 40 and two sublines of 'Waimanalo', Nos. 23 and 24. These three lines were significantly stunted by the disease and two of them significantly defoliated. Lines 45-T_{22} and 'Kapoho' which had intermediate mortality were severely defoliated and stunted. 'Higgins' the most susceptible

line had the highest mortality, it was also severely defoliated and stunted.

After transplanting survivors of inoculations along with uninoculated seedlings of the same line or hybrid into an infested field, further assessments of growth were made.

Line 8 did not seem to be affected adversely in growth in plant height or stem diameter. 'Waimanalo'-24 which exhibited growth reduction at the nursery stage recovered and appeared equal to the control 3 months after transplanting. This also occurred with 'Waimanalo'-23 by the 4th month after transplanting. Survivors of Line 40, a line considered resistant because of low mortality, remained stunted 4 months after transplanting compared to the control. Survivors of lines $45\text{-}T_{22}$ and 'Higgins' never recovered and grew unsatisfactorily.

In spite of the growth reduction found in some lines, it was observed that most, if not all surviving plants were bearing fruits as well as the control plants. This suggests that survivors from artificial inoculations performed at early stages of growth are able to reach production and are therefore capable of producing progenies which could be used for further selection.

APPENDIX

TABLE 35. -- Comparison of regression equations of percent mortality on concentration of inoculum of Line 40 and 'Higgins'

Papaya lines	Regression coefficient	Regression SS	Dev. from Regression SS	df	Total SS
40	28.3	67,433.3	9,766.7	11	77,200.0
Higgins	24.1	48,792.9	9,720.0	11	58,512.8
Pooled error SS			19,486.7	22	
Regression fitted to combined data	26.2	115,473.9	20,238.9	23	135,712.8

$$F = \frac{20{,}238.9 - 19{,}486.7}{23 - 22} \div \frac{19{,}486.7}{22} = 0.85 \text{ n.s.}$$

TABLE 36. -- Comparison of regression equations of percent mortality on concentration of inoculum of seedlings of four different ages of 'Higgins'

Seedling ages	Regression coefficient	Regression SS	Dev. from Regression SS	df	Total SS
2-week old	49.0	73,230.5	52,335.6	17	125,566.1
1-month old	13.3	4,800.0	266.7	3	5,066.7
2-month old	6.8	4,334.4	1,543.4	8	5,878.0
3-month old	5.95	2,232.1	276.6	5	2,588.7
Pooled error SS			54,422.2	33	
Regression fitted to combined data	13.4	38,308.5	100,791.0	36	139,099.5
Comparison of all ages tested		$F = \frac{100{,}791.0 - 54{,}422.2}{36 - 33} \div \frac{54{,}422.2}{33} = 9.37$ **			
Comparison of 2 and 3-month old seedlings		$F = \frac{1{,}925.7 - 1{,}820.0}{14 - 13} \div \frac{1{,}820.0}{13} = 0.76$ n.s.			

TABLE 37. -- Comparison of regression equations of percent mortality on concentration of inoculum of three papaya cultivars inoculated one week after germination

Papaya cultivars	Regression coefficient	Regression SS	Dev. from Regression SS	df	Total SS
Waimanalo-23	19.7	6,937.1	435.3	7	7,372.4
Kapoho Solo	21.8	8,522.5	1,045.6	7	9,568.2
Higgins	25.4	11,670.9	659.6	7	12,330.5
Pooled error SS			2,140.5	21	
Regression fitted to combined data	22.3	26,830.5	2,440.6	23	29,271.0

$$F = \frac{\frac{2,440.6 - 2,140.5}{23 - 21}}{\frac{2,140.5}{21}} = 1.47 \text{ n.s.}$$

TABLE 38. -- Comparison of regression equations of percent mortality on concentration of inoculum of three papaya cultivars inoculated 2 weeks after germination

Papaya cultivars	Regression coefficient	Regression SS	Dev. from Regression SS	df	Total SS
Waimanalo-23	26.4	12,534.7	1,840.1	7	14,374.8
Kapoho Solo	30.1	16,300.3	1,778.6	7	18,078.9
Higgins	31.0	17,319.1	3,136.5	7	20,455.6
Pooled error SS			6,755.2	21	
Regression fitted to combined data	29.2	45,938.1	6,921.3	23	52,909.4

$$F = \frac{\frac{6{,}921.3 - 6{,}755.2}{23 - 21}}{\frac{6{,}755.2}{21}} = 0.34 \text{ n.s.}$$

TABLE 39. -- Comparison of regression equations of percent mortality on concentration of inoculum of 1 and 2-week old seedlings of three papaya cultivars

Papaya cultivars and ages	Regression coef.	Regression SS	Dev. from Regr. SS	df	Total SS
1-week old Waimanalo-23	19.7	6,937.1	435.3	7	7,372.4
2-week old Waimanalo-23	26.4	12,534.7	1,840.1	7	14,374.8
Pooled error SS			2,275.4	14	
Regression fitted to combined data	23.0	19,074.8	2,672.4	15	21,747.3
		$F = \frac{2,672.4 - 2,275.4}{15 - 14} \div \frac{2,275.4}{14}$		= 2.44 n.s.	
1-week old Kapoho Solo	21.8	8,522.5	1,045.6	7	9,586.2
2-week old Kapoho Solo	30.1	16,300.3	1,778.6	7	18,078.9
Pooled error SS			2,824.2	14	
Regression fitted to combined data	25.9	24,197.9	3,449.2	15	27,647.1
		$F = \frac{3,449.2 - 2,824.2}{15 - 14} \div \frac{2,842.2}{14}$		= 3.10 n.s.	
1-week old Higgins	25.4	11,670.9	659.6	7	12,330.5
2-week old Higgins	31.0	17,319.1	3,136.5	7	20,455.6
Pooled error SS			3,796.1	14	
Regression fitted to combined data	28.2	28,712.2	4,073.9	15	32,786.1
		$F = \frac{4,073.9 - 3,796.1}{15 - 14} \div \frac{3,796.1}{14}$		= 1.02 n.s.	

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