EVALUATION OF F_2 POPULATIONS OF CROSSES AMONG RESISTANT, INTERMEDIATE AND SUSCEPTIBLE PAPAYA LINES FOR *PHYTOPHTHORA* ROOT ROT RESISTANCE

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Delfino Parra-Gutierrez

Thesis Committee:

H. Y. Nakasone, Chairman
R. W. Hartmann
M. Aragaki

We certify that we have read this thesis and that in our opinion it is satisfactory in scope and quality as a thesis for the degree of Master of Science in Horticulture.

THESIS COMMITTEE

Henry Y. Nakasone
Chairman

Richard W. Hartmann

M. Aragaki

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INTRODUCTION

For many years Hawaiian farmers have been unable to crop the same land to papaya without experiencing high mortality, decline in plant vigor and reduced yields due to the presence of a soil pathogen, particularly, Phytophthora palmivora (Butl.) Butl. (Nakasone and Aragaki, 1973) (Fig. 1).

Figure 1. Papaya orchard showing the replant problem in Malama-Ki, Hawaii

This pathogen causes root, stem and fruit rots of papaya. Parris (1941) first reported this disease causing damage at Aiea Heights, Kailua, and Kanoehe, Oahu. He pointed out that this disease possessed all the characteristics of a virulent disease and could become an important factor in the future of papaya production because it was able to attack fruits, stems, leaves and roots.

In 1971, Ko reported that more than 4,000 acres of papaya land in Hawaii had been abandoned due to the root rot disease.

In 1979, papayas occupied fourth place among the principal diversified Hawaiian crops with a value of $9,510,000 from 2,210 acres (Hawaii Agr. Rep. Serv., 1980).

Because of the high value of the crop and the extensive losses caused by <u>Phytophthora palmivora</u>, a breeding program was started in the early 1960's at the Waimanalo Experiment Station Farm on Oahu (Nakasone and Aragaki, 1973). Using 60 available lines and accessions, selection was conducted against the root rot problem during six continuous replantings in the same field. This study indicated that some lines such as Line 34 and 'Higgins' which were never subjected to replanting tests, showed high mortality rates, while others like 'Waimanalo' showed high tolerance with low mortality and good vigor under field conditions.

Nakasone (1976) mentioned that in field studies difficulties were encountered with concentration and distribution of the pathogen in the soil. Thus low mortality in a given line did not necessarily mean resistance unless accompanied by adequate numbers of replications. Mosqueda-Vazquez (1977) showed correlation between percent mortality obtained by inoculating one month old seedlings with a uniform concentration of inoculum to obtain 70% mortality under nursery conditions and mortality obtained under field conditions.

By this inoculation method Mosqueda-Vazquez (1977) classified the following lines as resistant to root rot: Line 8, 'Waimanalo'-23, 'Waimanalo'-24, and Line $40F_5$; as moderately resistant (intermediate): Line $45F_6T_{22}$ and 'Kapoho Solo'; and as susceptible: the cultivar 'Higgins'. He used the half diallel crossing system to estimate heritability of the progenies of the parental lines and found a highly significant additive genetic variance for the root rot resistance in the F_1 population.

Mosqueda-Vazquez (1977) found that this greenhouse inoculation method showed a correlation coefficient value of r = 0.9355** with percent mortality registered in the field and mean percent mortality in the greenhouse. He also found in his greenhouse studies a correlation coefficient of r = -0.8521** between percent mortality and percent selectable plants, and r = -0.9475** between percent mortality and disease rating. This greenhouse inoculation method also permits the screening of large populations, and provides reliable results as index of the behavior of the plants in the field. Therefore, the greenhouse method of screening of the F_2 populations was used in this study.

Mosqueda's study (1977) was confined to parental lines and F_1 populations and did not entail segregating populations. Thus, the objectives of this study were: (1) to determine percent mortality in F_2 populations as an index of resistance; (2) to determine the segregation in the F_2 population for vigor ratings; (3) to determine the most resistant lines using percent selectable plants as the index of disease resistance; and (4) to assess growth reduction of seedlings caused by _P. palmivora_ using measurements of percent defoliation and plant height.

LITERATURE REVIEW

Root Rot Organism

Phytophthora palmivora (Butl.) Butl., the organism that causes papaya root rot, belongs to the Phycomycetes, Subclass Oomycetes and the Family Pythiaceae (Roberts and Boothroyd, 1975). Trujillo and Hine (1964) consider that two Oomycetes, P. parasitica Dast. (identity of papaya pathogen was corrected to P. palmivora by Tokunaga and Bartniki-Garcia, 1971) and Pythium aphanidermatum (Edson) Fitz. are the most common causal agents of papaya root rot in Hawaii. But most authors refer only to P. palmivora as the pathogen causing root rot disease at the seedling stage (Aragaki, 1975; Ko, 1971; Ko and Chan, 1974; Mosqueda-Vazquez, 1977; Nakasone, 1975; Nakasone, 1976; Nakasone and Aragaki, 1973; and Ramirez and Mitchell, 1975).

Classification and Characteristics of the genus Phytophthora

Phytophthora, a genus of plant pathogenic fungi, has 35 different species, including P. palmivora (Zentmyer, 1976). It has a hyaline mycelium, cottony white in the host parts, and nonseptate. The sporangia are hyaline, oval, and measure 40 to 60 x 27 to 35μ. The zoospores, 15 to 30 per sporangium, measure 7 to 12μ (Weber, 1973). The chlamydospore is spherical, 19 to 38μ, or oval, from 19 to 28 x 28 to 56μ (Marcley, 1967). Zoospores are ovoid, bluntly pointed, and fattened on one side at the anterior end (Hickman, 1970). The Oospores are thick, wrinkled walled, sexual spores (Gallegly, 1970).

The important forms of inoculum of P. palmivora are chlamydospores, oospores, sporangia, zoospores, or pieces of plant containing mycelia (Brasier, 1969a; Brasier,1969b; Doo, 1964; Hunter and Buddenhagen, 1969; Hunter and Kunimoto, 1974; Kliejunas and Ko, 1973; Knutson and Eide, 1961; Ko and Chan, 1974; Marcley, 1967; Mitchell, 1978; Mosqueda-Vazquez, 1977; and Ramirez and Mitchell, 1975), each of them performing a function in the disease syndrome.

P. palmivora chlamydospores are the most prevalent resting structure (Marcley, 1967), while sporangia (Fig. 2) are the most infective spore type to papaya seedlings (Ko and Chan, 1974), and each of these sporangia produce 16 small motile zoospores which are also infective units; oospore is mainly a resting spore (Brasier, 1969a).

Spore development and germination in the genus Phytophthora

Sporangium is defined by Blackwell (1949) as an organ of vegetative reproduction. Germination of sporangium of P. palmivora takes place in two ways. The direct method involves the production of germ tubes which occur very slowly, often requiring 12 hours to obtain a maximum of 50% germination at an optimum temperature with a narrow peak at 28°C. Indirect germination is rapid and requires only water

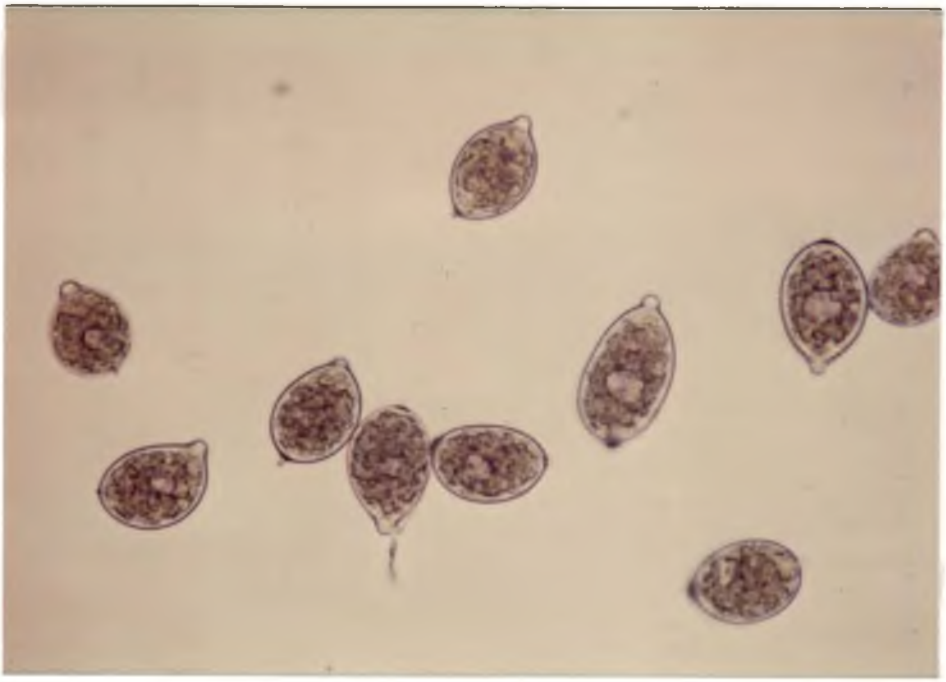

Figure 2. Sporangia of Phytophthora palmivora containing zoospores. (Courtesy of M. Aragaki, Plant Pathology Department, University of Hawaii)

to produce zoospores at temperatures from 16° to 31°C with a peak at 24° to 28°C. This process is almost completed in 30 minutes with 85% germination (Aragaki, et al, 1967; Brasier, 1969a).

Besides temperature and free water as factors that promote sporangial development and germination in the field and in the laboratory, there are other factors such as (1) changes in temperature as a pregermination treatment (Taylor,et al, 1955); (2) water potential between -0.3 and -4 bars (Duniway, 1975a); (3) 90 to 100% relative humidity (Hunter and Kunimoto, 1974; Trujillo, 1965); (4) flooded soil (Marcley, 1967); (5) papaya residues in field (Marcley, 1967; Trujillo and Hine, 1964 and 1965); (6) vegetable juice agar (in vitro) (Aragaki, 1975); and (7) light (Aragaki and Hine, 1974; Brasier, 1969a). Furthermore, there are some heavy metal cations that affect negatively the process of zoosporangial production and zoospore release of P. cinnamomi. Cations such as copper (Cu^{2+}), calcium (Ca^{2+}), and iron (Fe^{3+}) inhibited sporangial formation at concentrations between 1 and 5 x 10^{-4} mM' 100 and 1,000 mM, and 1 and 10 mM, respectively (Halsall, 1977; Halsall and Forrester, 1977).

Chlamydospores, as mentioned earlier, are the primary unit of survival for Phytophthora spp. in soil and roots. In heterothallic species like P. palmivora, chlamydospores play a more important role than oospores (Brasier, 1969b; Marcley, 1967; Zentmyer, 1976; Zentmyer and Erwin, 1970). The infection potential of chlamydospores of P. palmivora is intermediate between sporangia and zoospores, while the colonization potential of zoospores and chlamydospores is about the same (Ko, 1974). In Phytophthora, chlamydospores germinate in media typically by producing many germ tubes or, in some circumstances, by producing sporangia (Zentmyer and Erwin, 1970). Populations of chlamydospores of P. palmivora are increased 4 to 5 times after one week of incubation in beakers containing papaya plants (Ramirez and Mitchell, 1975).

Oospores of P. palmivora, heterothallic species with compatibility types A1 and A2 (Chee et al, 1976; Gallegly, 1970; Zentmyer and Mitchell, 1970; Zentmyer et al, 1973), are in general readily obtained in vitro when isolates of both compatibility types are jointly inoculated on a suitable agar medium and incubated at approximately 20°C in the dark. They are also formed on detached leaves of Piper nigrum when both compatibility types are inoculated and incubated at 15° to 27.5°C in the dark (Brasier, 1969a and 1969b).

Zoospores, according to Roberts and Boothroyd (1975), are swarm naked, motile spores produced within a sporangium. Indirect germination of P. palmivora sporangia may occur in the soil if water is available but with no nutrient source (Marcley, 1967). In this type of germination, the protoplasm of the sporangium is first segmented into zoospores which are liberated when they are fully mature and motile. Approximately 80% or more of the sporangia produce zoospores in the temperature range of 16° to 31°C, with the optimum at 24° to 28°C (Aragaki, et al, 1967). Zoospores of P. palmivora are the less effective spores as far as infecting papaya seedlings is concerned (Ko and Chan, 1974; Ramirez and Mitchell, 1975). These zoospores can be adversely affected or killed if they are placed in papain or freshly collected latex at concentrations as low as 10 to 20 ppm., but if they are allowed to encyst before treatment, they require approximately 250 to 500 ppm. of papain for inactivation (Hine, et al, 1965).

Identification, distribution and host plants of organism

According to a review by Chee (1969), *P. palmivora* was first described in 1907 by Butler under the name of *Pythium palmivora*. In 1942 Parris used the name of *P. parasitica* as the causal agent to the newly discovered papaya disease in Hawaii. Later, Tokunaga and Bartniki-Garcia (1971) transferred the papaya organism as *P. palmivora* and has since been used to describe the disease attributed earlier to *P. parasitica*.

Chee (1969) cited that in 1907 *P. palmivora* was pathogenic to *Cocos nucifera* and two other crops in India. In the following years the pathogen was reported in an increasing number of hosts, and by 1969 over 138 host plants were reported to be diseased by this pathogen, occurring in 21 different countries, located mostly in tropical and subtropical regions (Chee, 1969).

Symptoms of the disease

When the collar and roots of the trees are attacked by this pathogen, the stem is anchored less securely in the soil than is normally the case. Infected plants usually show a wilting, yellowing and premature falling of leaves. Much of the root system is affected and plants are blown down readily by even moderate winds. Sometimes the plant slowly recovers and continues to grow with the stem in a semi-recumbent position. The diseased collar and root parts are usually sloughed off from the plant (Parris, 1941 and 1942). Seedlings show high mortality and symptoms such as lack of vigor and defoliation (Aragaki, 1975; Ko, 1971; Mosqueda-Vazquez, 1977; Nakasone, 1976; Trujillo and Hine, 1964).

Factors involved in disease development

Significant factors affecting disease development include soil moisture, soil drainage, soil texture, soil temperature, papaya residues, osmotic and matric potential, and the phenomenon of chemotaxis.

The interactions between disease development and soil moisture, soil drainage and temperature are the most important ones. *Phytophthora* root rot is severe in infested soils with excessive soil moisture around the roots of susceptible plants and develops rapidly in trees growing in warm, poorly drained soils, under humid conditions (Roberts and Boothroyd, 1975). When *P. palmivora* is widely present in the soil as reported by Dakwa (1974), irrigation water is one way to spread this fungus over large areas (Taylor, 1977).

Dispersal of *P. palmivora* is increased through wind-blown rain droplets carrying sporangia on leaves, stem and fruits. When bigger drops are formed, large amounts of sporangia are dispersed (Hunter and Kunimoto, 1974).

Temperature plays a significant role in disease development. The optimum temperature for laboratory growth of P. palmivora is 28°C, while those for leaf and fruit infection are 26°C and 28°C, respectively (Doo, 1964). Soil temperatures are also important for disease development (Waggner and Shaw, 1953). In pineapple a soil temperature of around 30°C will promote optimum growth of the plant, but this is also optimum for P. parasitica growth in roots and crowns. On the other hand, if the soil temperature is around 19°C, severe root and heart rot will be caused by P. cinnamomi, which thrives at lower temperatures (Hine, et al, 1964).

Temperature responses are also useful in forecasting outbreaks of late blight on tomatoes (Harrison,1947), Phytophthora blight of taro (Trujillo, 1965), and P. parasitica on papaya fruits (Hunter and Buddenhagen, 1969).

Papaya crop residues, such as stem and fruits or old trees in abandoned fields, can act as the inoculum source and cause root and fruit rot, as well as mortality and growth inhibition of seedlings in replant orchards (Hunter and Buddenhagen, 1969). This is particularly the case during wet and windy weather, conditions which are conducive to release of sporangia from mycelial mats on infected fruits and stems in old fields (Hunter and Buddenhagen, 1969) or buried in the soil (Murashige, et al, 1965; Trujillo and Hine, 1964; Trujillo and Hine, 1965).

Moreover, factors such as depth, soil texture, and matric and osmotic potential of the soil can influence the active movement of the zoospores, indirect germination of the sporangia, or the growth of the fungus. Zoospores of _P. cryptogea_ in the surface water of flooded soils were able to swim 25 to 35 mm, while zoospore movement, when placed at 3 to 8 mm below the surface of flooded soils, was noticeably reduced, except when using U. C. type soil mix (Duniway, 1976). A similar situation occurred when soil matric potential was equal to -1 or -10 mb in Columbia silt loam, Yolo fine sandy loam, and Yolo clay loam, except on U. C. type soil mix in which zoospores moved commonly 25 mm at all matric potential values tested. On the other hand, indirect sporangial germination was reduced significantly at matric potential values of -1, -10, -50, and -300 mb (Duniway, 1976).

Sterne, et al (1977a and 1977b), working with _Phytophthora_ root rot of _Persea indica_, found that in soils in which matric potential was zero and no salt was added to the soil solution, 80 to 90% of the roots were diseased. Following reduction in matric potential to -0.05 and -0.10 bar, the percentage of diseased roots decreased to 60 to 85% and 10 to 50%, respectively.

Osmotic potential, on the other hand, reduced the growth of _P. cinnamomi_ in liquid media, but the disease rating on _Persea indica_ seedlings was not significantly different over the range of osmotic potentials used (Sterne, et al, 1976;

Sterne, et al, 1977).

One significant phenomenon in disease production is chemotaxis shown by the zoospores. Papaya roots exude some chemicals that attract zoospores of _P. palmivora_ in water within a minute; subsequently, the roots get covered by masses of encysted zoospores (Kliejunas and Ko, 1973).

Accumulation and encystment of _P. capsici_ in vitro occurs in the region of elongation immediately behind the root tips and over wounds which are the sites of maximum exudation of one or more compounds that stimulate zoospore accumulation (Hickman, 1970). Chemicals like vitamins, phenolic compounds, nitrogenous bases of nucleic acid, nucleotides, growth regulators, sugars, organic acid, and amino acids gave positive stimuli to zoospores of five _Phytophthora_ species, including _P. palmivora_. Moreover, it was found that there are differential chemosensitive responses of the five _Phytophthora_ species to some amino acids and that the ionic structure of the amino acid molecule is important in determining its chemotactic activity (Khew and Zentmyer, 1973).

Phytophthora root rot-host relations

Plants attacked by pathogens can respond necrotically, hypoplastically, or hyperplastically to the activities of the pathogen. Toxins and hydrolytic enzymes often are responsible for the necrotic reactions of the diseased plants (Roberts and Boothroyd, 1975).

Host plant roots are damaged by the action of *Phytophthora*, thereby reducing the manufacturing capability of roots (Labanauskas, et al, 1975). Infection of avocado seedling roots by *P. cinnamomi* decreased the dry weight of roots by 86%, stem by 88%, and leaves by 82%, as compared to values obtained from uninfected plants. The fungus not only affected the dry weight, but also influenced adversely the nutrient concentration found in the roots, stem, and leaves of infected plants (Labanauskas, et al, 1976).

Besides the reduction in the root system, Duniway (1975 and 1977) found that root disease of safflower caused by *P. drechsleri* increased resistance to water uptake through the root system and also greatly increased the resistance to water movement through the xylem in stems. Resistance of infected roots and stems was more than 8 and 40 times the respective resistance in healthy plants. He also pointed out that nearly half the resistance to water flow through infected plants was located in the xylem containing mycelium and other foreign materials.

Sterne, et al (1978) hypothesized that *P. cinnamomi* in avocado induced changes in the water relations of avocado trees that resulted in severe plant water stress that resembled stress caused by low soil water potential.

Several important parameters were altered considerably in infected trees: leaf xylem pressure potential was lower during the day and night than in healthy trees, probably a primary factor inducing stress symptoms. These low values indicate that even when transpiration was zero, insufficient water moved from the soil to the leaves to permit recovery during the night. They also stated that xylem pressure potential of infected trees was not coupled with transpirational flux, while in healthy trees, xylem pressure potential increased or decreased with transpiration. These studies indicate that _P. cinnamomi_ induced such major changes in the soil-to-leaf water transport system that typical physiological controls of water loss in plants could not correct the water deficits.

Keen et al (1975) found that mycolaminarans (water soluble β-1, 3-glucans) from _P. cinnamomi_, _P. palmivora_ and _P. megasperma_ behave as phytotoxins and produce wilting symptoms on _Persea indica_, soybean, cacao, and tomato at 0.01 to 0.5 mg./ml. The plant responses to mycolaminarans appeared to be caused by cellular toxicity rather than by vessel plugging.

Roberts and Boothroyd (1975) mention some responses of host plants to pathogens: (1) some plants have a hypersensitive necrotic reaction, a defensive response of the suscept to the pathogen actions which is so sudden and complete in the vicinity of the invading pathogen, that the pathogen can not survive due to the failure to establish food relationship with the host. This kind of reaction stimulates the production of phytoalexins and also increases the activity of polyphenol oxidase within attacked cells, products which turn the cells brown or black and then cause them to die as a defensive response of the plants; (2) another response is the formation of cellular or gummous demarcations to the pathogenic attack which limites the pathogen or its spread.

Control of the pathogen

Root rot control in orchards is largely a matter of prevention. Up to now no satisfactory and economical method of eradicating the fungus from the soil has been reported (Roberts and Boothroyd, 1975). There are several reports in which plants in soil treated with fumigants and fungicides showed better growth (Hamilton, 1965; Lange, 1960; Murashige and Nakano, 1965; Trujillo and Hine, 1964), but in one case, under poorly drained soils of Waimanalo, Oahu, no beneficial growth response of papaya was found on land treated with several soil fumigants (Lange, 1960).

Biological control of papaya seedling root rot can be done (Ko, 1971) by planting papaya seeds in small quantities of pathogen-free virgin soil placed in planting holes in infested fields. The principle involved is to protect young susceptible seedlings, one and two months old, from infection. After three months the plants develop resistance to P. palmivora. Later, Mosqueda-Vazquez (1977) found that in papaya some time after the second week after germination, resistance begins to develop and is substantial in one and two month old seedlings.

Another possible source of biological control is the use of culture of microorganisms like the Actinomycetales that exhibit potential antagonism to several Pythium and Phytophthora spp. Some cultures of this group appear to be highly active against some isolates of Phytophthora (Knauss, 1976).

Development of resistance

According to Day (1974), there are two kinds of resistance: (1) Non-host resistance exhibited by wheat to the potato late blight pathogen; and (2) Host resistance resulting from the genotype of the host, which gives resistance to pathogens that would otherwise grow on the host plant. There are four types of host resistance in plants (Holtzmann, O. V. verbal communication, 1980): hypersensitivity, escape, tolerance, and immunity.

Whichever type of resistance is present in the host plant, the basis for resistance would be (1) morphotological, which results from structures that prevent the entrance of a pathogen; (2) protoplasmic, in which the protoplast cell of the host plant prevents the pathogen from becoming established in the host tissue (biochemical resistance), and (3) functional, in which the functioning of the plant gives resistance to invasion by the pathogen such as in the case of wheat resistance to stem rust in which the stomata opens only for a short period around noon, a time which is not favorable for the fungus (Roberts and Boothroyd, 1975).

Whatever the basis of resistance, the inheritance may be oligogenic, polygenic, and cytoplasmic. Oligogenic resistance is determined by one or a few genes, and the individual effects are quickly observed; polygenic resistance is determined by many genes whose individual effects are small; cytoplasmic resistance is determined by the cytoplasm and its organelles; its importance is small in the general resistance spectrum (Day, 1974).

Determination of resistance to plant pathogenic organisms presents many variables. Methods used in testing resistance to root rotting organisms like <u>Phytophthora</u> include: growing and selecting plants in naturally infested soil (Nakasone and Aragaki, 1973; Zentmyer, 1976); dipping papaya roots in spore suspension (Aragaki, 1975); growing avocado plants in nutrient solution and adding the pathogen in one or several forms (Zentmyer and Mircetich, 1965); or screening of one month old papaya seedlings by inoculating the medium with sporangia suspension, tested to produce 70% mortality (Mosqueda-Vazquez, 1977).

The main variables involved besides method of inoculation of seedlings are age of the seedlings and concentration of the inoculum used (Doo, 1964; Hine, et al, 1965; Knutson and Eide, 1961; Ko, 1971; Mosqueda-Vazquez, 1977; Nakasone and Aragaki, 1973). As far as both factors are concerned, Mosqueda-Vazquez (1977) found that papaya seedlings grown in peat pots with vermiculite as the growing medium, showed a definite relationship between age of the seedlings, concentration of inoculum and susceptibility to papaya root rot. Younger papaya seedlings are more susceptible to root rot and low concentrations of inoculum are sufficient to produce 100% mortality.

In exploring the basis for high resistance in Persea spp. Zentmyer (1976) cites from Zaqui et al (1973, 1976) that several wild species contain borbonol which is toxic to Phytophthora as well as to other fungi. This chemical first isolated from Persea borbonia is either not present or found only in small amounts in susceptible avocado.

MATERIALS AND METHODS

Materials

Papaya seeds used in the study of F_2 populations for root rot resistance were chosen from Mosqueda-Vazquez's (1977) work in which he used a 5 x 5 half diallel crossing method between five parental inbred lines, namely, Line $40F_5$, 'Waimanalo'-23, 'Waiamanalo'-24, Line $45F_6T_{22}$ and 'Higgins'. The following F_1 lines were used to produce the F_2 population: (crosses were classified according to Mosqueda-Vazquez, 1977):

Resistant x Resistant

1. $314AF_2$ (Line $40F_5$ x 'Waimanalo'-23)
2. $314BF_2$ (Line $40F_5$ x 'Waimanalo'-24)
3. $317F_2$ ('Waimanalo'-23 x 'Waimanalo'-24)

Resistant x Intermediate

4. $315F_2$ (Line $40F_5$ x Line $45F_6T_{22}$)
5. $316AF_2$ ('Waimanalo'-23 x Line $45F_6T_{22}$)
6. $316BF_2$ ('Waimanalo'-24 x Line $45F_6T_{22}$)

Resistant x Susceptible

7. $341F_2$ (Line $40F_5$ x 'Higgins')
8. $318AF_2$ ('Waimanalo'-23 x 'Higgins')
9. $318BF_2$ ('Waimanalo'-24 x 'Higgins')

Intermediate x Susceptible

10. $319F_2$ (Line $45F_6T_{22}$ x 'Higgins')

11. 'Higgins' was used as the susceptible control line to determine the mortality level of the inoculum concentration used in this study.

The experiment was set up as a randomized complete block design with 11 treatments consisting of 10 F_2 papaya progenies lines and 1 control line. Five replicates were used, each replication consisting of 30 seedlings.

Methods

Seedling culture and the method of inoculation used in this study followed the method described by Mosqueda-Vazquez (1977), which allows the screening for root rot disease of large populations of papaya seedlings in a limited area.

This method is briefly described as follows: Seeds were germinated in community pots, using No. 2 vermiculite under greenhouse conditions (Fig. 3). Germination occurred between 9 to 20 days after sowing.

At about the 20th day after seeds were sown, the seedlings were transplanted into individual 8 cm. depth peat pots (Fig. 4 and 5), using vermiculite as the growing medium. Daily watering and weekly fertilization was done with a 0.3% solution of a 19-19-19 fertilizer, containing minor elements such as B, Mo, Mn, Fe, Cu, Mg, and Zn at an

Figure 3. Community pots filled with No. 2 vermiculite

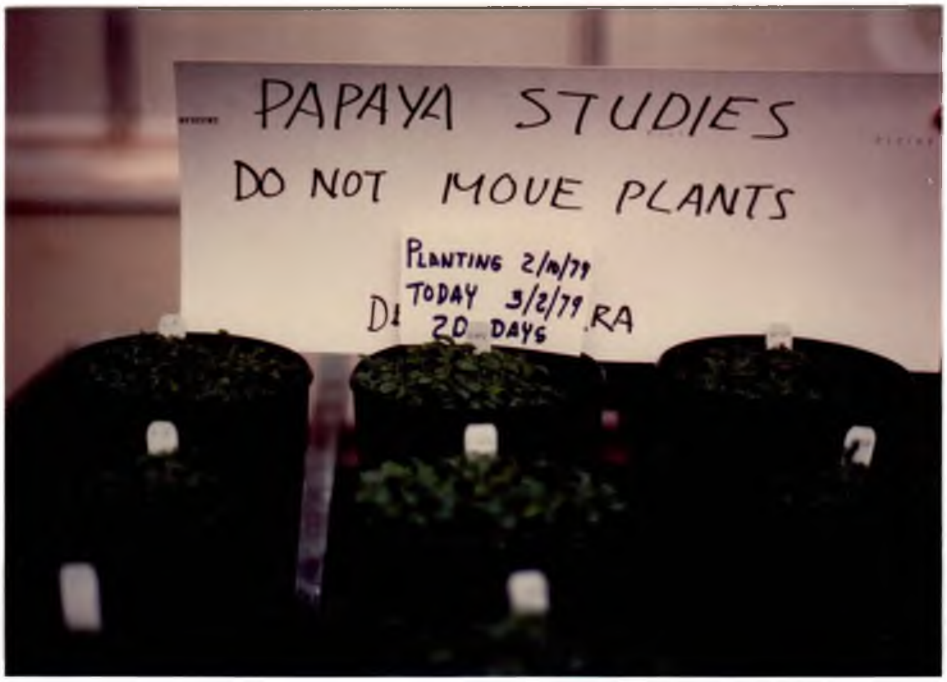

Figure 4. Papaya seedlings at the cotyledonary leaf stage ready for transplanting into individual peat pots at the 20th day after inoculation

approximate rate of 30 ml. per plant until one month after transplanting into the peat pots, at which time they were ready for the inoculation treatment (Figs. 6, 7, 8 and 9). Fertilization was withheld on the week of the inoculation, and then was resumed the following week at the same weekly basis until field transplanting of survivors a month later.

Inoculation of the seedlings was done when they were one month old, using a uniform concentration of sporangia per plant from P268 culture of *Phytophthora palmivora* obtained from the Plant Pathology Department. Cultures of P268 were grown as described by Aragaki (1975) on vegetable juice agar (10% Campbell's V-8 juice, 0.2% $CaCO_3$) in petri dishes under continuous Cool White fluorescent irradiation (approximately 2700 lx.) at 24°C for six days in order to produce abundant sporangia. The sporangia from 24 petri dishes were brushed off into deionized water at 24°C. Estimates of the number of sporangia per ml. were made by counting as many fields of a hemacytometer required to get at least a total of 200 sporangia in about 8 to 10 fields and then calculating to get the approximately concentration of sporangia per ml. in the suspension. Dilutions were made as necessary on the basis of estimated number of sporangia in the original suspension (Fig. 10). When seedlings were ready for inoculation, they were watered in the morning and inoculated in the afternoon.

Figure 5. Peat pots in flats with capacity of 30 individual 8 cm. depth units

Figure 6. Papaya seedlings, 1 week after transplanting into individual peat pots

Figure 7. Papaya seedlings, 2 weeks after transplanting into individual peat pots

Figure 8. Papaya seedlings, 3 weeks after transplanting into individual peat pots

Figure 9. Papaya seedlings, 4 weeks after transplanting into individual peat pots. Ready for inoculation with 3800 sporangia/plant of P. palmivora

Figure 10. Inoculation materials such as P. palmivora sporangia solution in the beaker, 10ml. pipette, 25ml. dispenser and a funnel

Twenty-five ml. of the sporangial suspension was required to provide approximately 3800 sporangia per plant.

In this experiment the P268 isolate of *P. palmivora* was used because the virulence of the P170 isolate used by Mosqueda-Vazquez (1977) was greatly reduced. Therefore, there was the need to conduct a series of four trials initially in order to determine the required concentration of inoculum of the new P268 isolate to cause approximately 80% mortality in 'Higgins', the susceptible control line.

A dispenser was used to pour the inoculum directly into each pot (Fig. 11). A funnel (Fig. 12) was used to prevent spillage of the suspension on the leaves or stems of the papaya seedlings. One month after inoculation, surviving seedlings were drenched with approximately 24 ml. of a suspension of Truban 30W fungicide at 250 ppm. to arrest the disease. Truban is a systemic fungicide composed of ETMT 30% (ETMT = 5-Ethoxy-3-trichloromethyl-1, 2, 4-thiadiazole).

Variables measured

The variables used in the present study were selected from Mosqueda-Vazquez's (1977) work, particularly those which gave significant results to assess the degree of resistance of the papaya lines studied, and those which helped to evaluate the differences between lines to measure the extent to which growth was reduced by root rot.

Figure 11. Filling in the 25 ml. dispenser with inoculum solution of P. palmivora

Figure 12. Pouring 25 ml. of 3800 sporangia per plant inoculum solution with the aid of a funnel to avoid splashing of inoculum to the stem

In the assessment of the degree of resistance of the F_2's to root rot, three variables were measured:

1. Percent mortality. A plant was considered dead when it had two or less fully expanded leaves at the apex of the seedling (Fig. 13).
2. Vigor rating. This was based on disease symptoms and rated on a scale of 1 to 5 in which 1 = dead plant, when only two expanded leaves remained (Fig. 14); 2 = weak plant with chlorotic leaves and approximately 75% defoliation (Fig. 14); 3 = plant with intermediate vigor and approximately 50% defoliation (Fig. 15); 4 = vigorous plant with 25% defoliation and few chlorotic leaves (Fig. 16); and 5 = vigorous plant with no apparent symptoms above ground (Fig. 17).
3. Percent selectable plants. Plants with vigor rating of 4 and above (Fig. 18) were considered acceptable for commercial plantings.

These three variables were measured in all plants throughout the experimental units. Percentages were transformed to angles to stabilize the variance, where angle = arc sin $\sqrt{\%}$ (Snedecor and Cochran, 1967). Transformed percent mortality, vigor ratings and transformed percent selectable plants were analyzed by an analysis of variance. The three variables mentioned above were measured four weeks after inoculation.

Figure 13. Papaya seedlings classified as dead, when the number of leaves was reduced to two or less

Figure 14. Vigor rating of seedlings; 1=dead plant; 2=weak plant with approximately 75% defoliation

Figure 15. Vigor rating of seedlings: 3=plant with intermediate vigor and approximately 50% defoliation

Figure 16. Vigor rating of seedlings: 4=vigorous plant with approximately 25% defoliation

Figure 17. Vigor rating of seedlings: 5=vigorous plant with no apparent symptoms above ground

Figure 18. Percent selectable plants, includes those rated 4 and above

The selected variables used to detect growth differences between lines induced by the effects of the disease were: percent defoliation (Fig. 19), and plant height in centimeters (Fig. 20) from the cotyledonary node to the stem apex (Mosqueda-Vazquez, 1977). These two variables were measured when seedlings were ready to be inoculated at one month of age, and again at one month after inoculation. Both variables were measured in all plants.

Analyses of variance were conducted on the following data:

1. Percent defoliation--this was calculated using the equation:

$$\% \text{ D} = \frac{(\text{Ll}_{1-2})100}{\text{El}_2}$$

   where % D = percent defoliation; Ll_1 = number of leaves counted just prior to inoculation and Ll_2 = number of leaves counted one month after inoculation; El_2 = expected number of leaves present at the end of the second month of growth, which was taken from their respective F_2 uninoculated control seedlings.

2. The increase in plant height was calculated by subtracting the height before inoculation from the height one month after inoculation.

The data taken at the first and second month of age were recorded individually for all plants. Data taken at

Figure 19. Number of true leaves counted at one and two months old papaya seedlings

Figure 20. Height from cotyledonary node to the stem apex measured at one and two months old

the second month of age were taken only for the plants that survived the inoculation. Exception was on percent defoliation in which data from dead plants were also included in the analysis as plants with two leaves because by a previous definition they were considered dead when only two fully-expanded leaves remained attached to the plant.

Tests of significance were done using the Duncan's Bayesian Least Significant Difference test ("BLSD") to assess the level of resistance and to evaluate the extent to which growth of the F_2 population was reduced by the *P. palmivora* root rot.

RESULTS AND DISCUSSION

Percent mortality

Percent mortality obtained from 10 F_2 lines and the control 'Higgins' was higher than those obtained by Mosqueda-Vazquez (1977) in the F_1 population because higher concentration of sporangia per plant was used in this study. Consequently, the selection intensity was increased to a higher level. According to Falconer (1960), the rate of response to selection can be improved, and the selection process may be accelerated by increasing the selection intensity. Mosqueda-Vazquez (1977) suggested that in papaya, it is possible to increase the selection intensity based on the linear relationship between concentration of inoculum and percent mortality. Falconer (1960) also states that when increasing the selection intensity, the breeder should be aware about factors such as population size and inbreeding that can affect the breeding program.

Figures 21 and 22 show symptoms of yellowing of the leaves, wilting, and mortality of the inoculated F_2 papaya seedlings at the second and fourth week after inoculation with 3800 sporangia of *P. palmivora* per plant.

At the fourth week, percent mortality in the F_2 population ranged from 24.6 in $317F_2$ to 81.9 in $319F_2$ (Table 1, 2). Significant differences were observed between $319F_2$ which showed the highest percent mortality and $317F_2$ which showed the lowest percent mortality. These

Figure 21. Papaya seedlings, 2 weeks after inoculation with 3800 P. palmivora sporangia concentration per seedling, showing yellowing of leaves

Figure 22. Papaya seedlings, 4 weeks after inoculation with 3800 P. palmivora sporangia concentration

Table 1. Mean percent mortality obtained one month after inoculation in F_2 papaya population of seedlings inoculated at one month of age

Lines	% mortality Transformed arc sine $\sqrt{\%}$	Original %
'Higgins' (control, susceptible line)	70.42a	87.9
$319F_2$ (Line $45F_6T_{22}$ x 'Higgins')	65.92ab	81.9
$318AF_2$ ('Waimanalo'-23 x 'Higgins')	55.79bc	67.9
$315F_2$ (Line $40F_5$ x Line $45F_6T_{22}$)	55.17bc	65.9
$316BF_2$ (Line $45F_6T_{22}$ x 'Waimanalo'-24)	52.72c	62.6
$341F_2$ (Line $40F_5$ x 'Higgins')	50.52c	59.3
$316AF_2$ ('Waimanalo'-23 x Line $45F_6T_{22}$)	47.35cd	54.0
$318BF_2$ ('Waimanalo'-24 x 'Higgins')	46.91cd	53.3
$314AF_2$ (Line $40F_5$ x 'Waimanalo'-23)	37.37de	37.3
$314BF_2$ (Line $40F_5$ x 'Waimanalo'-24)	31.98e	31.3
$317F_2$ ('Waimanalo'-23 x 'Waimanalo'-24)	28.40e	24.6

CV = 19.86%

[z]Mean separation by BLSD test, 5% level

differences were expected inasmuch as $319F_2$ is the product of a cross between intermediate and susceptible parents while $317F_2$ resulted from a cross between two resistant parents. These results are in agreement with the analysis of parental and F_1 data reported by Mosqueda-Vazquez (1977). Table 1 shows the presence of three groups for root rot resistance in the F_2 population studied which are statistically different: (1) resistant group formed by $317F_2$, $314BF_2$ and $314AF_2$ populations which involve only resistant parents; (2) intermediate group formed by $318BF_2$, $316AF_2$, $341F_2$, $316BF_2$, $315F_2$, and $318AF_2$ populations which involved resistant parents crossed with intermediate and susceptible parents, and (3) susceptible group formed by $319F_2$, which involves an intermediate parent crossed with a susceptible one.

The data in Table 1 have been reconstructed in Table 2 to show more clearly the contributions to the mean percent mortality by the different parental lines. 'Waimanalo'-24 as a parent line gave better gain in resistance to P. palmivora root rot in crosses with 'Higgins' and Line $40F_5$ than 'Waiamanalo'-23 did in similar crosses. On the other hand resistance is slightly improved when 'Waimanalo'-23 is the parent line with Line $45F_6T_{22}$ over two other resistant parental lines, 'Waimanalo'-24 and Line $40F_5$

Mosqueda-Vazquez (1977) detected the presence of a significant additive variance in study of the F_1 population

Table 2. Comparison of mean percent mortality obtained one month after inoculation in F_2 papaya population of seedlings inoculated at one month of age

	Mean percent mortality values of F_2 seedlings arranged by Parents[x]			
	'Waimanalo' 23 (R)[z]	'Waimanalo' 24 (R)	Line $45F_6T_{22}$ (I)	'Higgins' (S)
Line $40F_5$ (R)[z]	37.3de	31.3e	65.9bc	59.3c
'Waimanalo'-23 (R)		24.6e	54.0cd	67.9bc
'Waimanalo'-24 (R)			62.6c	53.3cd
Line $45F_6T_{22}$ (I)				81.9ab
			(Control)	87.9a

CV = 19.86%

[x]Mean separation by BLDS test, 5% level on transformed arc sine $\sqrt{\%}$ values

[z](R) resistant line; (I) intermediate resistant line; and (S) susceptible line

of papaya line crosses. This is one of the three recognized components of the hereditary variance as reported by Allard (1960), and it is useful in the estimation of the effects of gene action, since additivity implies the shift on the scale if one allele is substituted by another, regardless of the presence of other genes.

Vigor rating analysis

Rating of plant vigor is another genetic index in detecting levels of resistance to P. palmivora root rot.

Vigor ratings taken one month after inoculation showed that the mean vigor rating of 1.62 in the F_2 population of Line $40F_5$ x Line $45F_6T_{22}$ (resistant x intermediate) fell in the same statistical range as those of 'Higgins' and the F_2 Line $45F_6T_{22}$ x 'Higgins' (intermediate x susceptible) (Table 3). The cross between resistant and intermediate lines was expected to have higher resistance than a cross between intermediate and susceptible lines. This suggests that the allelic contribution of Line $40F_5$, a resistant parent, was of a small order or that its genetic interaction was a negative one inasmuch as the mean rating of this cross was expected to be higher than that of a cross between resistant and susceptible lines where additive variance prevailed. The presence of nonallelic interaction with genes of Line $45F_6T_{22}$ suppressing the genes for resistance from Line $40F_5$ may be a possibility. This suppressing

Table 3. Mean vigor rating obtained one month after inoculation in F_2 papaya population of seedlings inoculated at one month of age

Lines	Disease rating^x
'Higgins' (Control, susceptible line)	1.18a^y
$319F_2$ (Line $45F_6T_{22}$ x 'Higgins')	1.30ab
$315F_2$ (Line $40F_5$ x Line $45F_6T_{22}$)	1.62abc
$316BF_2$ (Line $45F_6T_{22}$ x 'Waimanalo'-24)	1.73bc
$318AF_2$ ('Waimanalo'-23 x 'Higgins')	1.76bc
$318BF_2$ ('Waimanalo'-24 x 'Higgins')	1.86c
$341F_2$ (Line $40F_5$ x 'Higgins')	1.88cd
$316AF_2$ ('Waimanalo'-23 x Line $45F_6T_{22}$)	2.11cde
$314BF_2$ (Line $40F_5$ x 'Waimanalo'-24)	2.42def
$314AF_2$ (Line $40F_5$ x 'Waimanalo'-23)	2.45ef
$317F_2$ ('Waimanalo'-23 x 'Waimanalo'-24)	2.84f

CV = 24.47%

^{x}Vigor rating from 1 = dead plant, to 5 = healthy plant

^{y}Mean separation by BLDS test, 5% level interaction was also suggested in previous work in the F_1 population reported by Mosqueda-Fazquez (1977).

The F_2 population of Lines $40F_5$ x Line $45F_6T_{22}$, $45F_6T_{22}$ x 'Waimanalo'-24, 'Waimanalo'-23 x 'Higgins', 'Waimanalo'-24 x 'Higgins', Line $40F_5$ x 'Higgins', and 'Waimanalo'-23 x Line $45F_6T_{22}$ fall more or less in the same statistical range of means, forming an intermediate group (Table 3). The resistant lines appear to show additive effects in the population of crosses in which they are involved. The mean vigor rating of the F_2 populations from crosses involving resistant x intermediate parents generally improved. This is further substantiated by the resistance in the populations among crosses between resistant lines, forming the group with the highest level of resistance.

The response in vigor of the different parental lines can be seen more clearly by arranging the ratings by their respective parents (Table 4). As in percent mortality, the best F_2 populations are 'Waimanalo'-23 x 'Waimanalo'-24, Line $40F_5$ x 'Waimanalo'-23, and Line $40F_5$ x 'Waimanalo'-24. It should be mentioned here that the mean vigor ratings in this study are lower than those presented by Mosqueda-Vazquez (1977), probably caused by the use of higher concentration of inoculum, imposing a higher level of selection intensity.

Similar types of interaction found in the mean percent mortality are also observed for mean vigor rating (Table 4). The cross 'Waimanalo'-23 x Line $45F_6T_{22}$ showed better

Table 4. Comparison of mean vigor rating obtained one month after inoculation in F_2 papaya population of seedlings inoculated at one month of age

	Mean vigor rating of F_2 seedlings arranged by Parents[x]			
	'Waimanalo' 23 (R)[z]	'Waimanalo' 24 (R)	Line $45F_6T_{22}$ (I)	'Higgins' (S)
Line $40F_5$ (R)[z]	2.45ef	2.42def	1.62abc	1.88cd
'Waimanalo'-23 (R)		2.84f	2.11cde	1.76bc
'Waimanalo'-24 (R)			1.73bc	1.86c
Line $45F_6T_{22}$ (I)				1.30ab
			(Control)	1.18a

CV = 24.47%

[x]Mean separation by BLSD, 5% level

[z](R) resistant line; (I) intermediate resistant line; and (S) susceptible line

combinging ability for disease resistance than Line $40F_5$ x $45F_6T_{22}$, and 'Waimanalo'-24 x Line $45F_6T_{22}$, although the means are not statistically different. Slight increased level of resistance in crosses involving resistant x susceptible were derived by the use of Line $40F_5$ and 'Waimanalo'-24 as the resistant parental lines (Table 4).

The frequency distribution histogram presented in Figures 23 through 33 are for the purpose of showing the distribution of the seedlings in the 10 F_2 populations and the susceptible, inbred line, 'Higgins', and are not intended to determine types of gene action leading to genetic ratio analysis; although it is possible to see in these histograms effects of F_2 segregation to the different rating categories, determination of genetic ratios is not possible in the population studied because of the high selection pressure imposed by inoculation with the number of sporangia calculated to produce approximately 80% mortality in the susceptible inbred population. Seedlings with moderate to even high disease tolerance may have succumbed to this selection pressure, producing a relatively skewed curve.

In Line $317F_2$ ('Waimanalo'-23 x 'Waimanalo'-24) 42% of the population were rated 4 or better (Fig. 23). These seedlings would all fall into the selectable group, acceptable for commercial plantings. The high selection intensity imposed upon the population resulted in 24.6% mortality.

The distribution range of Line $314AF_2$ (Line $40F_5$ x

'Waimanalo'-23) (Fig. 24) was similar to that of Line $317F_2$. There was a slight increase in the number of plant mortality (37.3%) and a decrease in the number of selectable plants (32%). The number of plants in the poor (rating of 2) and intermediate (rating of 3) groups are also similar to that of Line $317F_2$.

Plant mortality for Line $314BF_2$ (Line $40F_5$ x 'Waimanalo'-24) (Fig. 25) was slightly decreased as compared to that of Line $314AF_2$, a cross having similar parentage. However, there is a high increase in the number of plants in the number 5 group (17%) as compared to the distribution for Line $314AF_2$. However, the combined percentage of plants in ratings 4 and 5 were still lower than that of Line $314AF_2$.

The distribution pattern for Lines $316AF_2$ ('Waimanalo'-23 x Line $45F_6T_{22}$) (Fig. 26), $341F_2$ (Line $40F_5$ x 'Higgins') (Fig. 27), $318BF_2$ ('Waimanalo'-24 x 'Higgins') (Fig. 28), $316BF_2$ (Line $45F_6T_{22}$ x 'Waimanalo'-24) (Fig. 29), $315F_2$ (Line $40F_5$ x Line $45F_6T_{22}$) (Fig. 30), and $318AF_2$ ('Waimanalo'-23 x 'Higgins') (Fig. 31) were similar. They are characterized by increasing percent mortality and decreasing percent selectable plants.

A high increase in plant mortality is shown in the distribution range of Line $319F_2$ (Line $45F_6T_{22}$ x 'Higgins') (Fig. 32) resembling that of 'Higgins' (Fig. 33), the susceptible inbred line. Recovery of selectable plants was negligible.

Figure 23. Frequency histogram for the distribution of mean vigor rating obtained one month after inoculation of 317 F_2 ('Waimanalo'-23 x 'Waimanalo'-24) population of seedlings inoculated at one month of age

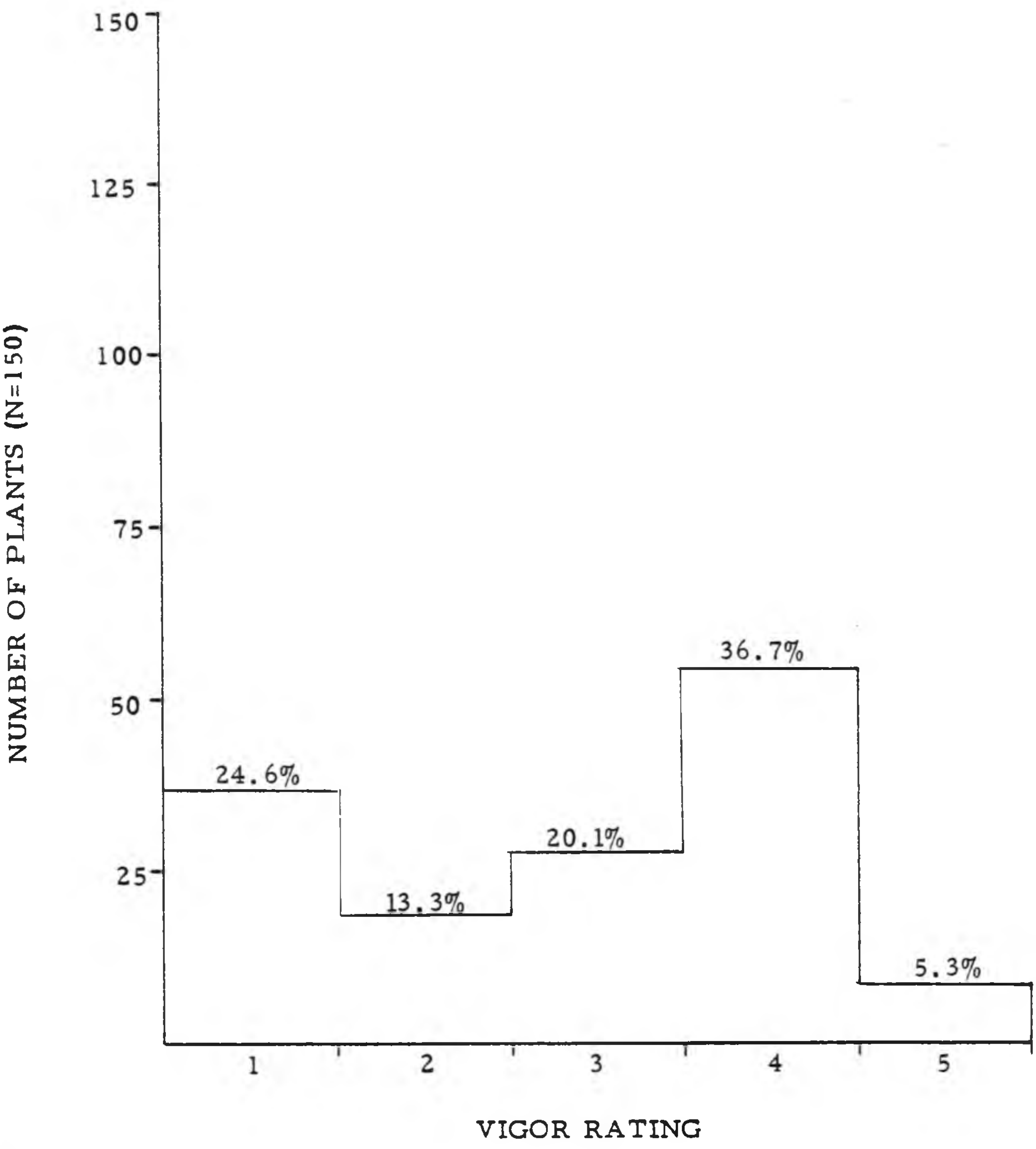

Figure 24. Frequency histogram for the distribution of mean vigor rating obtained one month after inoculation of 314AF_2 (Line 40F_5 x 'Waimanalo'-23) population of seedlings inoculated at one month of age

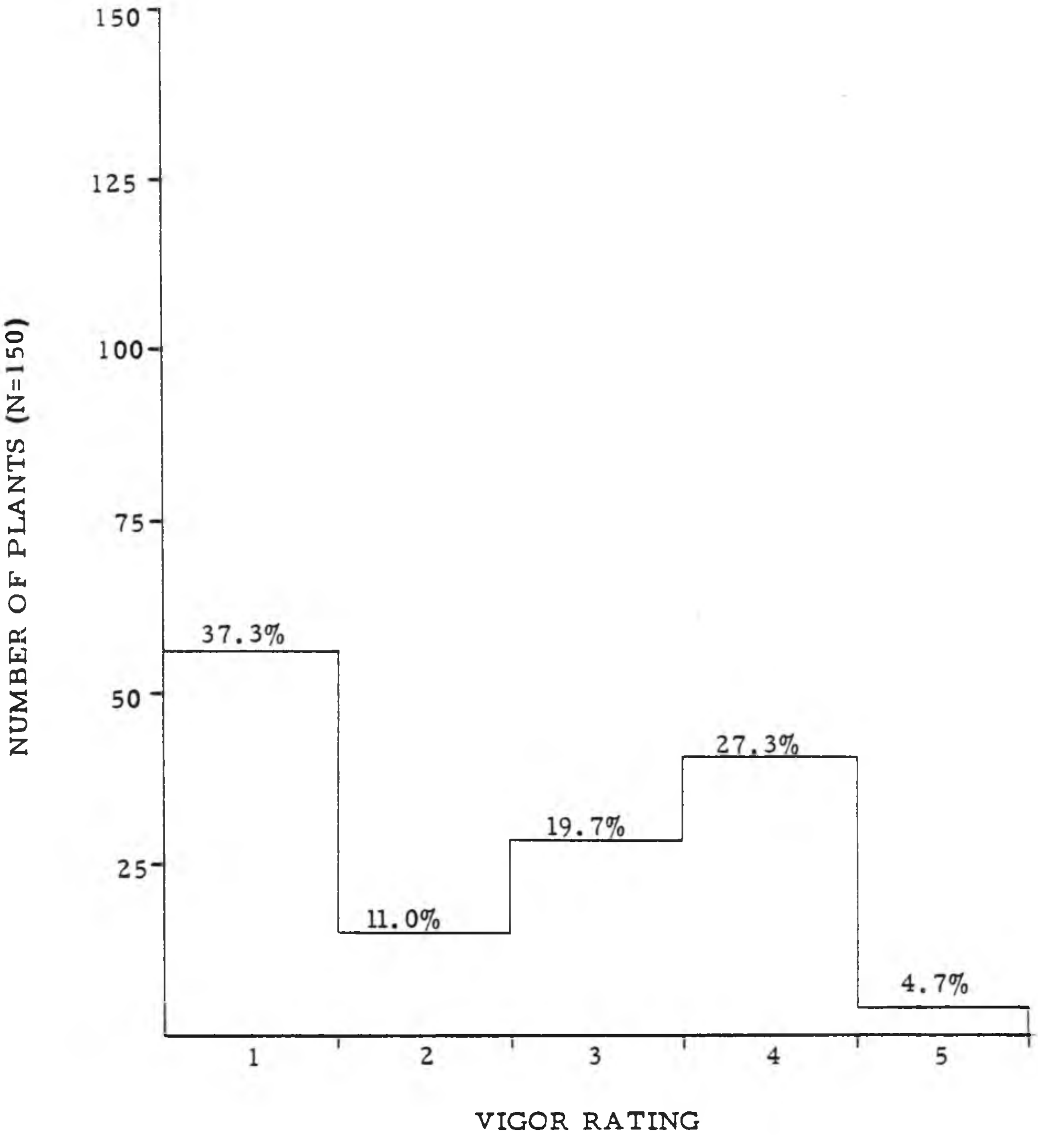

Figure 25. Frequency histogram for the distribution of mean vigor rating obtained one month after inoculation of 314BF$_2$ (Line 40F$_5$ x 'Waimanalo'-24) population of seedlings inoculated at one month of age

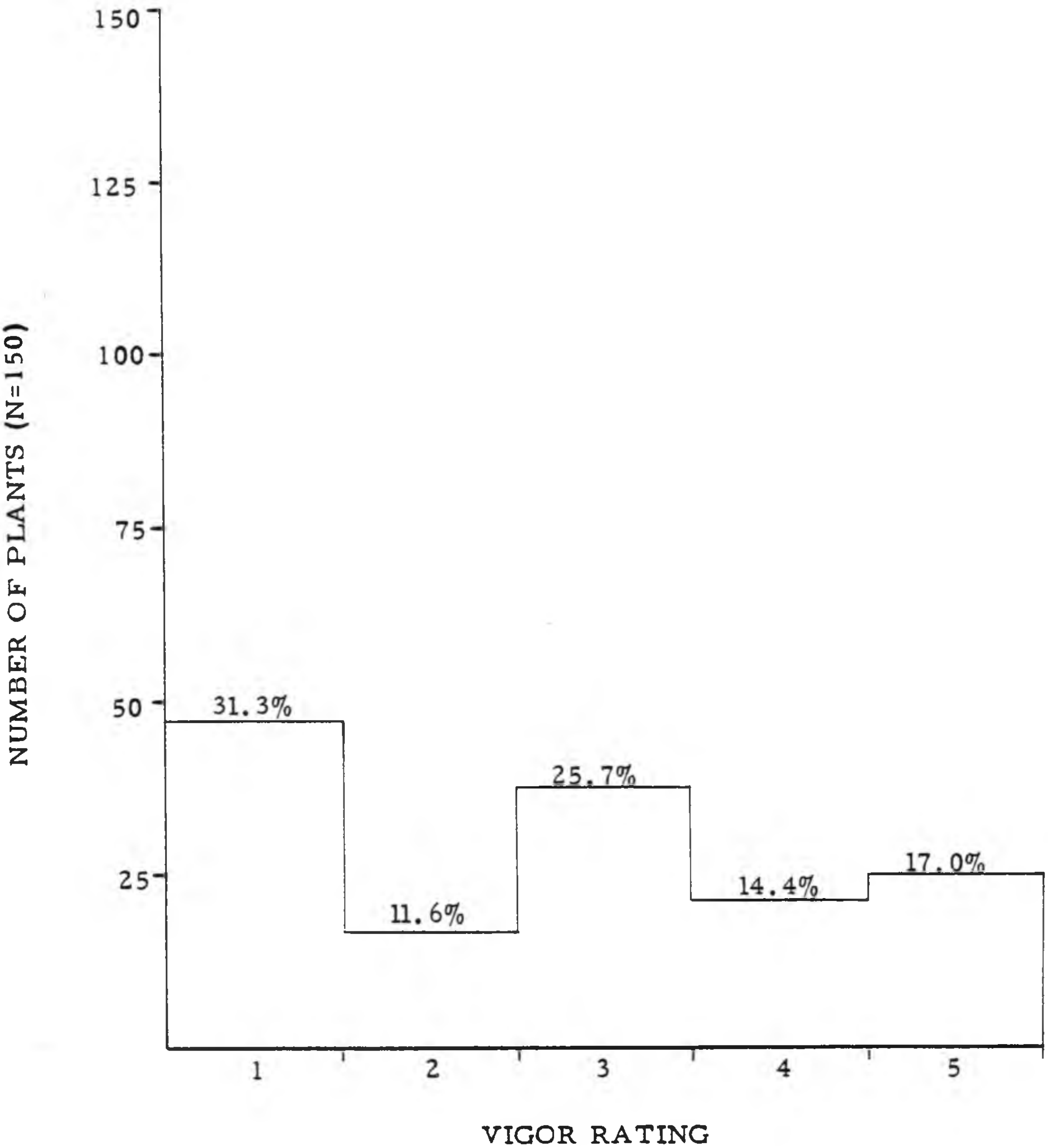

Figure 26. Frequency histogram for the distribution of mean vigor rating obtained one month after inoculation of 316AF_2 ('Waimanalo'-23 x Line 45F_6T_{22}) population of seedlings inoculated at one month of age

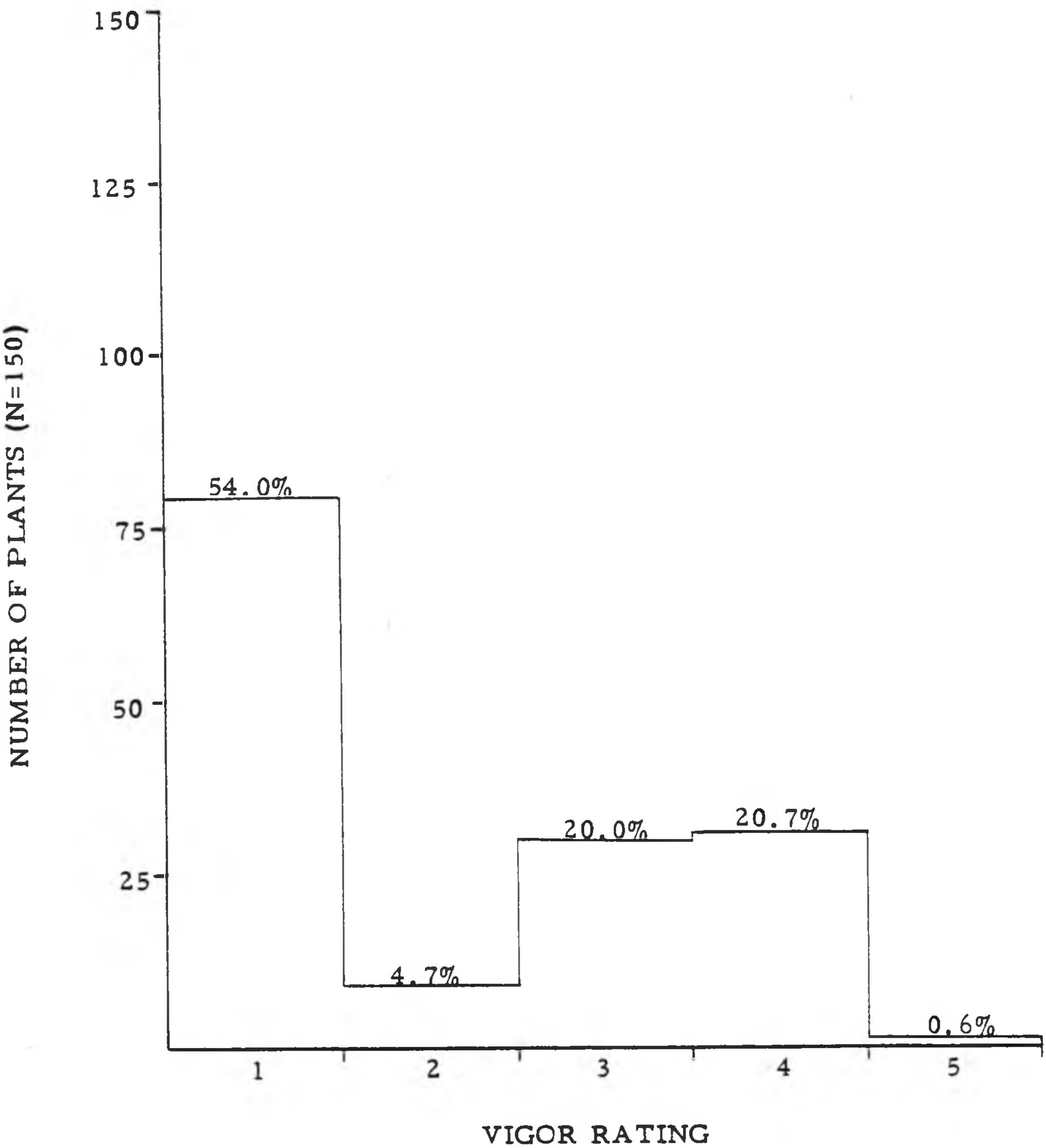

Figure 27. Frequency histogram for the distribution of mean vigor rating obtained one month after inoculation of $341F_2$ (Line $40F_5$ x 'Higgins') population of seedlings inoculated at one month of age

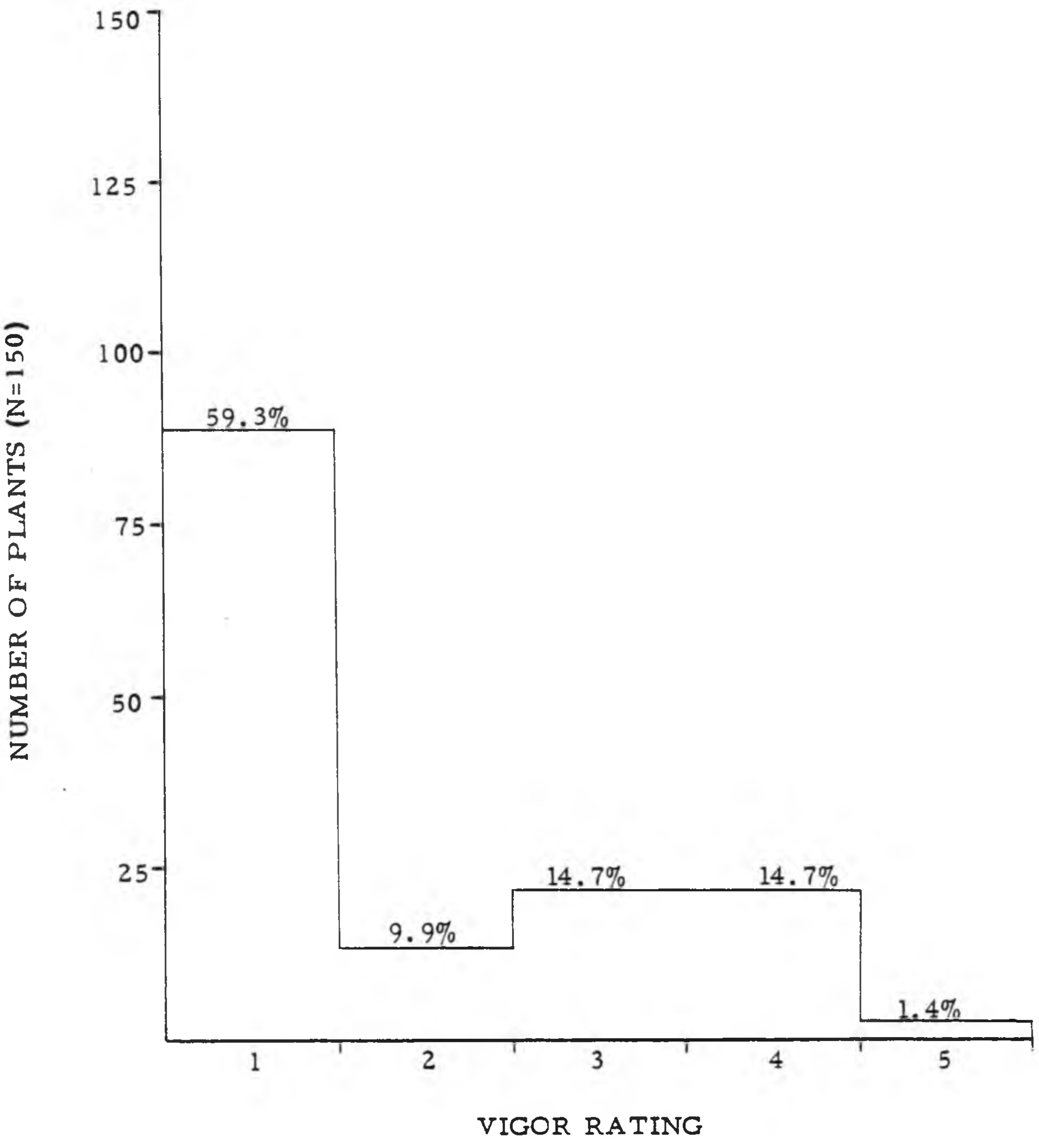

Figure 28. Frequency histogram for the distribution of mean vigor rating obtained one month after inoculation of 318BF_2 ('Waimanalo'-24 x 'Higgins') population of seedlings inoculated at one month of age

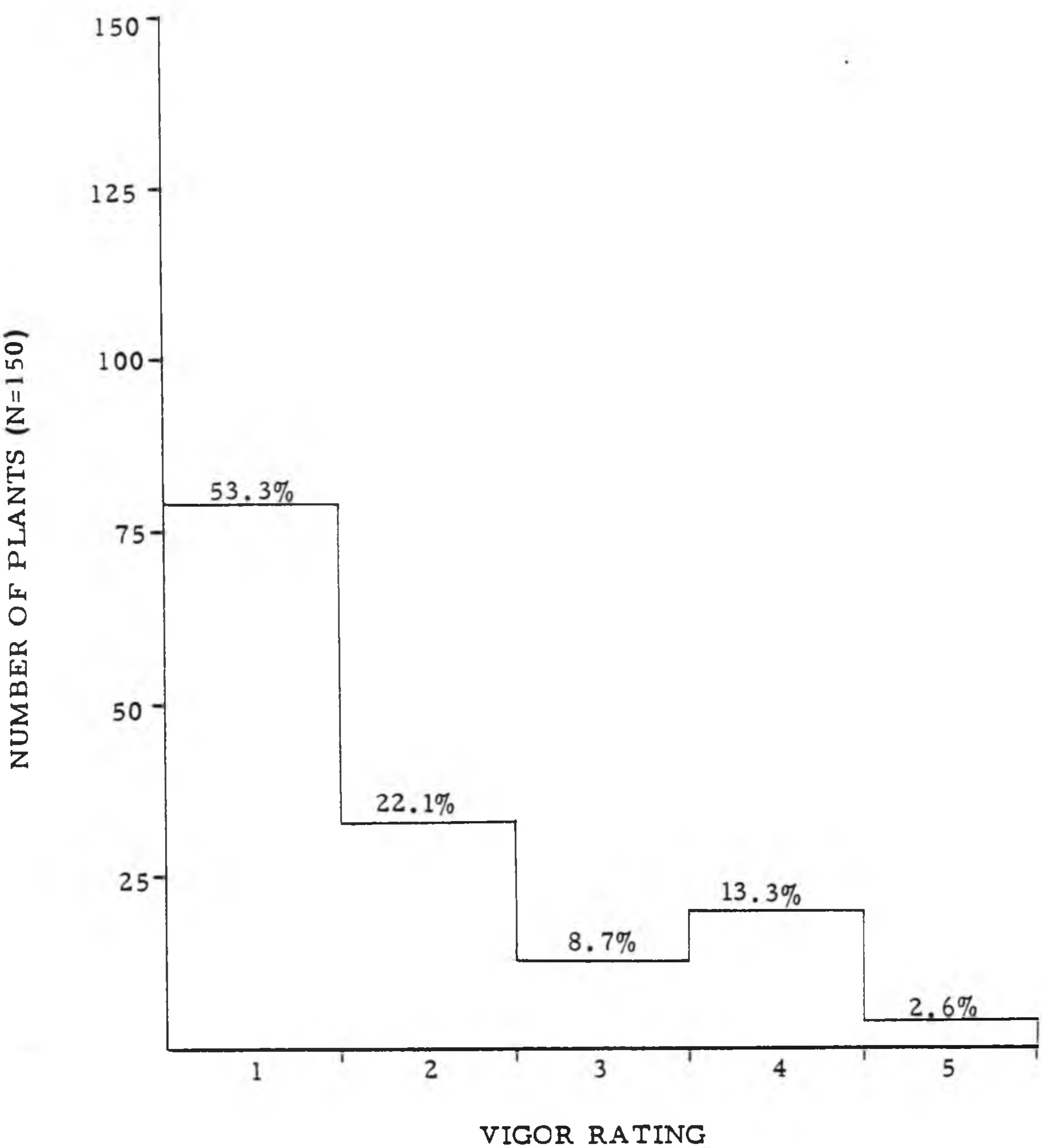

Figure 29. Frequency histogram for the distribution of mean vigor rating obtained one month after inoculation of $316BF_2$ (Line $45F_6T_{22}$ x 'Waimanalo'-24) population of seedlings inoculated at one month of age

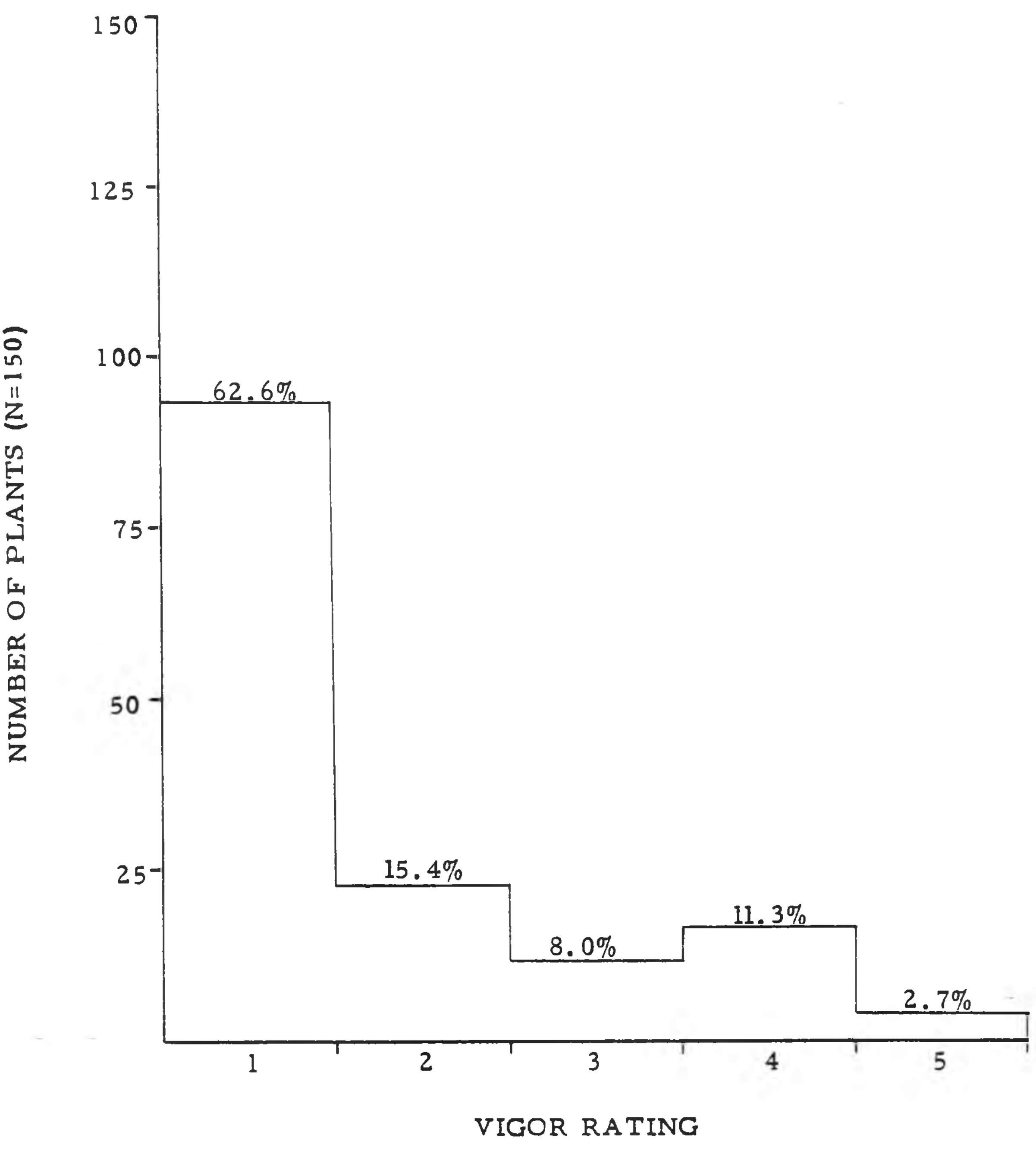

Figure 30. Frequency histogram for the distribution of mean vigor rating obtained one month after inoculation of $315F_2$ (Line $40F_5$ x Line $45F_6T_{22}$) population of seedlings inoculated at one month of age

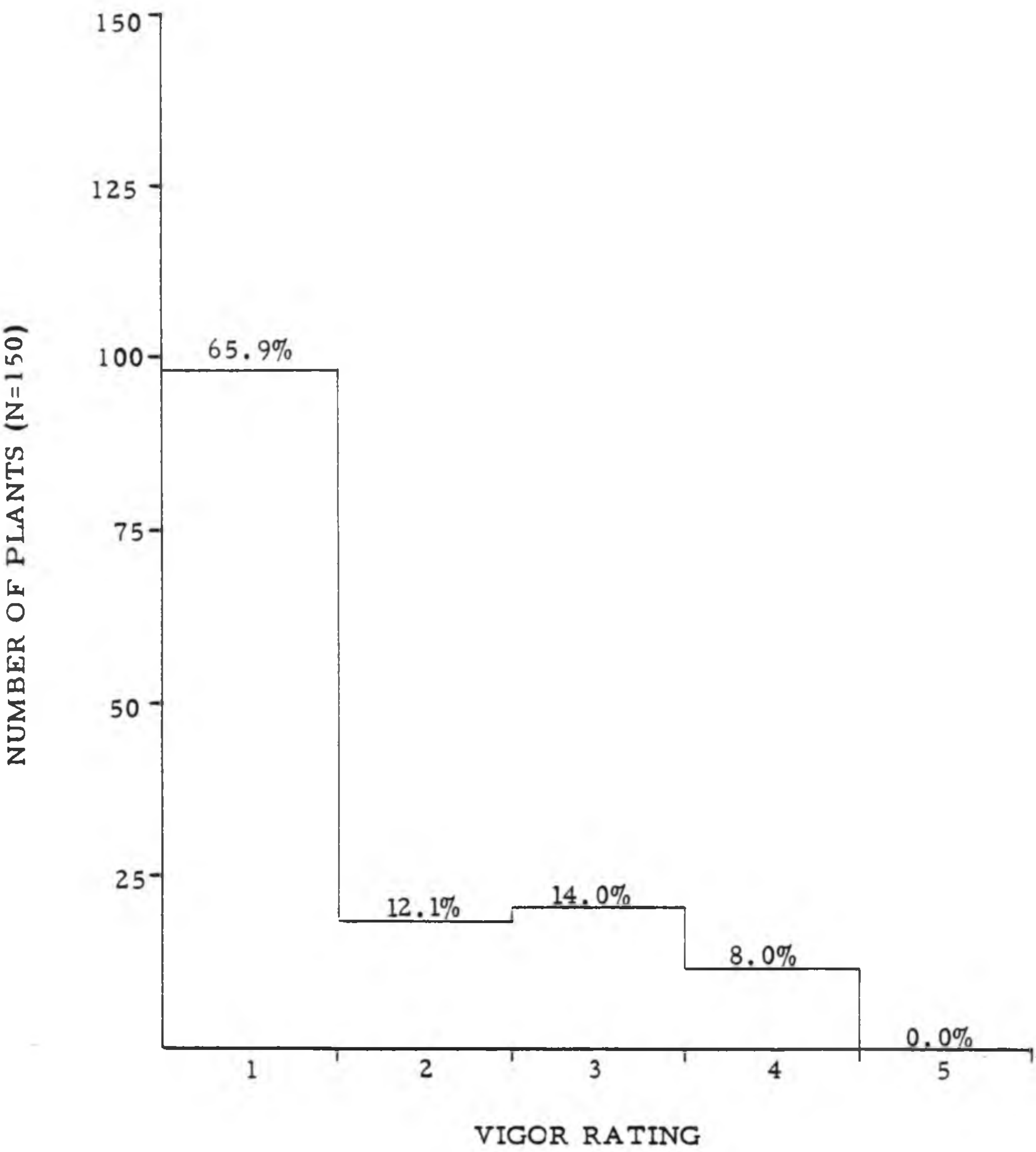

Figure 31. Frequency histogram for the distribution of mean vigor rating obtained one month after inoculation of 318AF_2 ('Waimanalo'-23 x 'Higgins') population of seedlings inoculated at one month of age

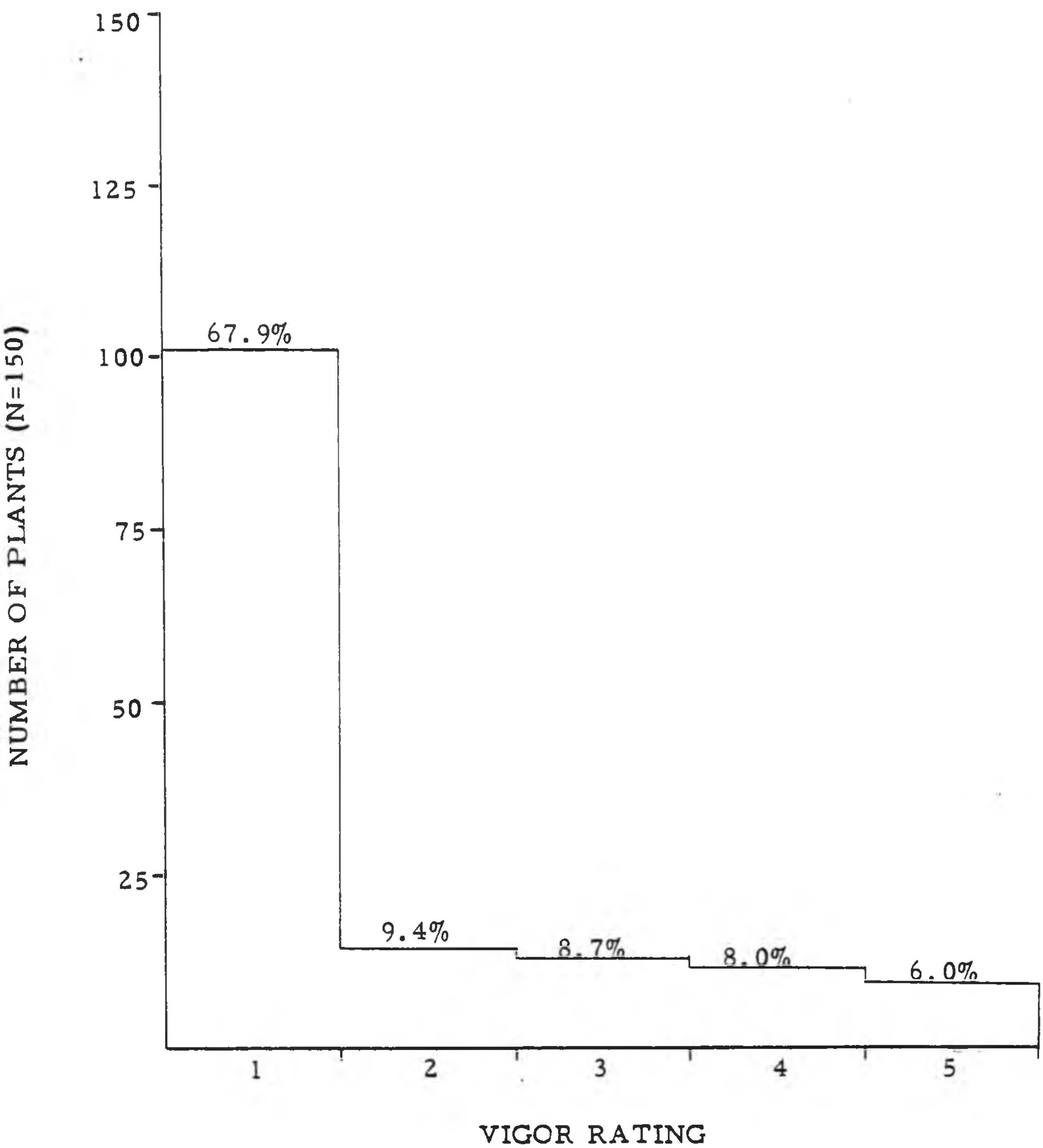

Figure 32. Frequency histogram for the distribution of mean vigor rating obtained one month after inoculation of $319F_2$ (Line $45F_6T_{22}$ x 'Higgins') population of seedlings inoculated at one month of age

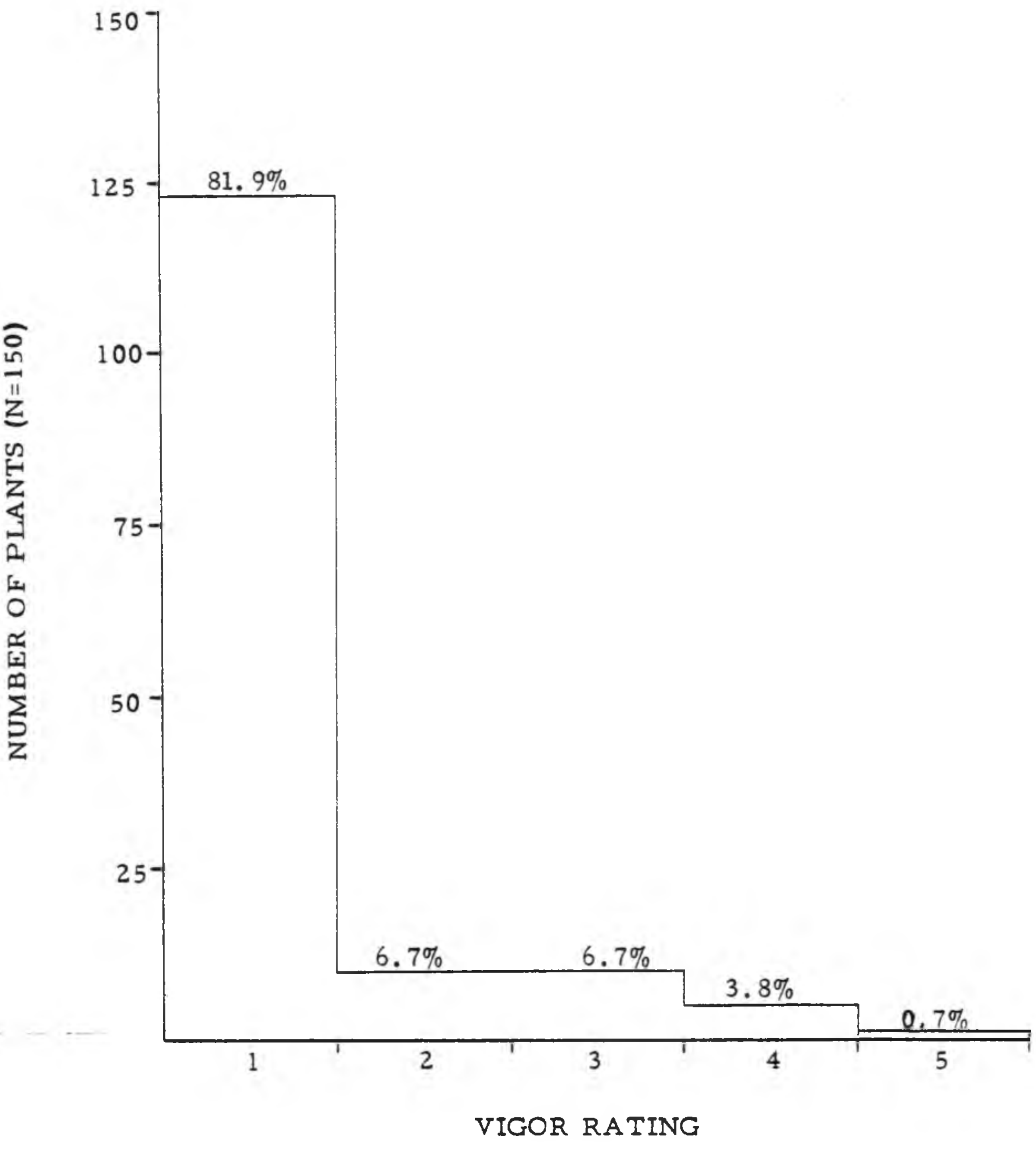

Figure. 33. Frequency histogram for the distribution of mean vigor rating obtained one month after inoculation of 'Higgins' (susceptible control line) population of seedlings inoculated at one month of age

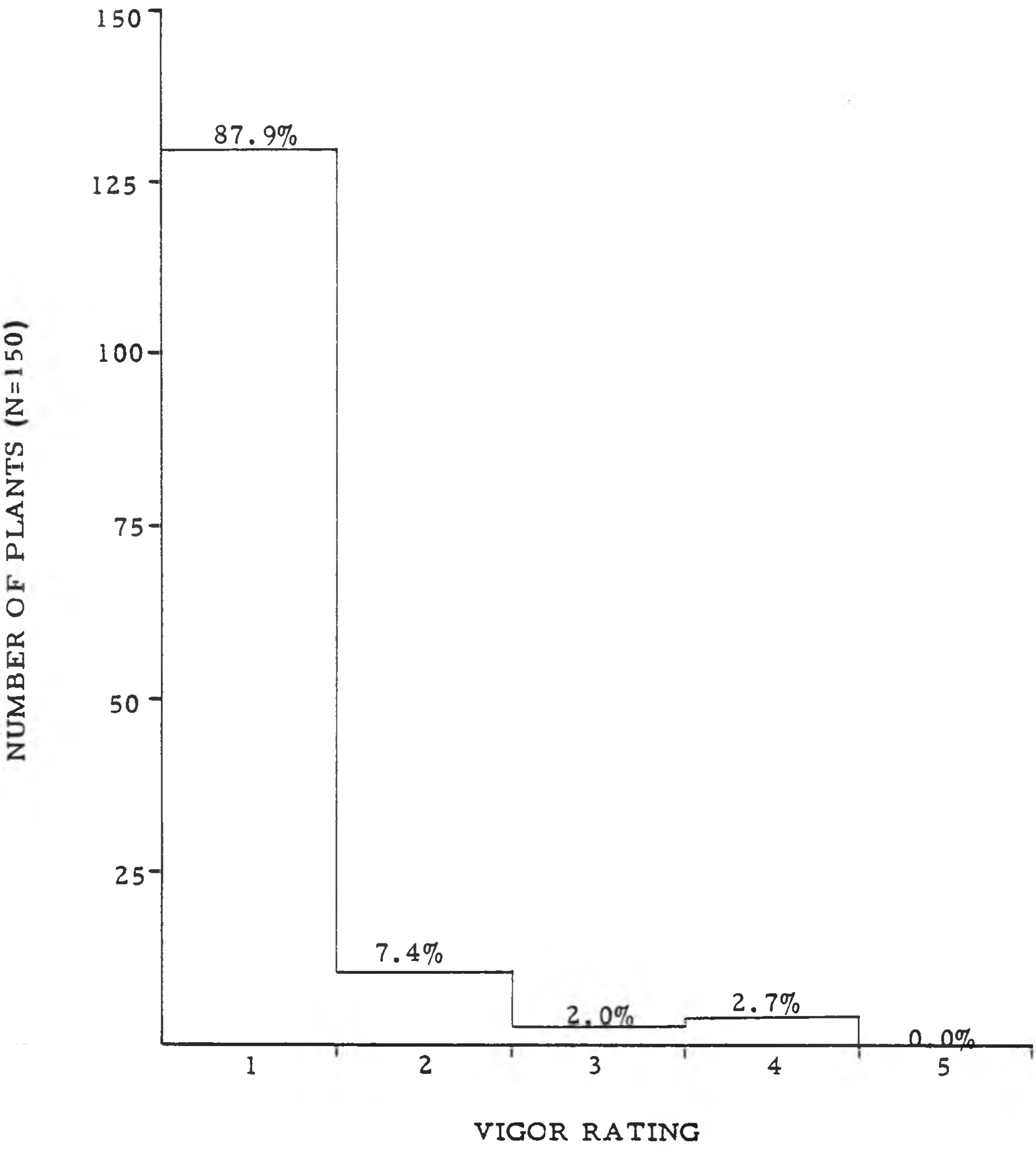

Table 5 presents the variances obtained for the different F_2 populations; these variances indicate: (1) the lowest variation was obtained in the homozygous line 'Higgins' which confirms the expected homozygosity of the line; (2) increased variation values were found in all the F_2 populations as compared with the homozygous line 'Higgins', suggesting that segregation to root rot resistance was present in the F_2 populations; consequently this result confirms that the F_2 generations were more variable than the parental line 'Higgins'; (3) variation of the $319F_2$ population is the lowest among all the F_2, which may suggest that the variation in this susceptible F_2 population is quite similar to that of the susceptible line due to the lack of resistance to papaya root rot; (4) the high variation presented in the group of resistant F_2 populations like $314BF_2$, $314AF_2$ and $317F_2$ suggests the presence of different genes in the parents involved for root rot resistance and other characters; consequently the segregation obtained produced the high variance value in the resistant populations.

Analysis of percent selectable plants

Selectable plants are those that are rated 4 and higher and are considered vigorous enough to be commercially accepted. Populations with a large number of selectable plants would be considered to possess a high degree of resistance. Rate of seedling mortality is an important parameter but equally important in determining resistance

Table 5. Variances in the F_2 populations for root rot resistance

Line	S^2*
314BF_2 (Line 40F_5 x 'Waimanalo'-24)	2.15
314AF_2 (Line 40F_5 x Waimanalo'-23)	1.83
317F_2 ('Waimanalo'-23 x 'Waimanalo'-24)	1.68
316AF_2 ('Waimanalo'-23 x Line 45F_6T_{22})	1.62
318AF_2 ('Waimanalo'-23 x 'Higgins')	1.57
341F_2 (Line 40F_5 x 'Higgins')	1.44
318BF_2 ('Waimanalo'-24 x 'Higgins')	1.39
316BF_2 (Line 45F_6T_{22} x 'Waimanalo'-24)	1.35
315F_2 (Line 40F_5 x Line 45F_6T_{22})	.99
319F_2 (Line 45F_6T_{22} x 'Higgins')	.68
'Higgins' (control, susceptible line)	.35

*S^2 = Variance

is the vigor of survivors. A population may exhibit a low mortality rate but survivors may be without vigor. In this variable it is possible to see that there is a small number of selectable plants, a situation that can be explained in terms of vigor ratings which were low because of the level of inoculation imposed.

As expected, 'Higgins' produced the lowest percentage of selectable plants. F_2 population from crosses involving 'Higgins' also showed relatively low percentage of selectable plants. The F_2 population of sibmated 'Waimanalo' and those of Line $40F_5$ crossed with the two 'Waimanalo' sublines gave the highest percent of selectable plants (Table 6).

The high coefficient of variation shown on Table 6 indicates that there is a high variation present in the population. This may be due to the variation present in the segregating F_2 populations or to the increase in the selection intensity used in this study, that reduced the number of potential plants that may have been rated 4 and above. Also in some F_2 susceptible populations there were no selectable plants in some replications. These were recorded as zero in the analysis of variance for percent selectable plants. Plants in the selective portion of the F_2 population may be the ones that inherited more genes for root rot resistance.

Table 6. Mean percent selectable plants obtained one month after inoculation in F_2 papaya population of seedlings inoculated at one month of age

Line	% Selectable plants[z] Transformed arc sine $\sqrt{\%}$	Original %
'Higgins' (Control, susceptible line)	7.17a	2.66
$319F_2$ (Line $45F_6T_{22}$ x 'Higgins')	11.35ab	3.99
$315F_2$ (Line $40F_5$ x Line $45F_6T_{22}$)	12.69abc	8.00
$316BF_2$ (Line $45F_6T_{22}$ x 'Waimanalo'-24)	20.74bcd	14.96
$318AF_2$ ('Waimanalo'-23 x 'Higgins')	21.06bcd	13.33
$341F_2$ (Line $40F_5$ x 'Higgins')	21.20bcd	16.00
$318BF_2$ ('Waimanalo'-24 x 'Higgins')	22.46bcd	16.00
$316AF_2$ ('Waimanalo'-23 x Line $45F_6T_{22}$)	25.38cd	21.33
$314BF_2$ (Line $40F_5$ x 'Waimanalo'-24)	32.05de	30.67
$314AF_2$ (Line $40F_5$ x 'Waimanalo'-23)	34.04e	32.00
$317F_2$ ('Waimanalo'-23 x 'Waimanalo'-24)	40.40e	42.66

CV = 47.27%

[z]Mean separation by BLSD, 5% level

Seedling growth analysis

Percent defoliation was used to assess the effect of root rot on the growth of the seedlings during the 30-day period after inoculation. Defoliation was higher in populations involving a parent with lower resistance to root rot than in populations involving parents with higher levels of resistance (Table 7). Statistical differences again separated the F_2 population into approximately three groups. An unexpectedly high 60.18% defoliation was observed in the F_2 population of the cross between Line $40F_5$ (classified as resistant) and Line $45F_6T_{22}$ (classified as intermediate) (Mosqueda-Vazquez, 1977). However, this result is consistent with previous reports in which Line $45F_6T_{22}$ was severely defoliated by the effect of the same disease (Mosqueda-Vazquez, 1977). The lowest percent defoliation was in $314BF_2$ (Line $40F_5$ x 'Waimanalo'-24) in which both parents are classified as resistant to root rot. Relatively high percent defoliation was shown by $341F_2$ (Line $40F_5$ x 'Higgins'), and $318AF_2$ ('Waimanalo'-23 x 'Higgins') with 52.47 and 51.32 percent defoliation, respectively. Intermediate percent defoliation was registered by $319F_2$ (Line $45F_6T_{22}$ x 'Higgins'), $318BF_2$ ('Waimanalo'-24 x 'Higgins'), $316AF_2$ ('Waimanalo'-23 x Line $45F_6T_{22}$), and $316BF_2$ (Line $45F_6T_{22}$ x 'Waimanalo'-24) with 50.18, 47.91, 44.16 and 38.83 percent, respectively. Lower levels of defoliation were recorded for $314AF_2$ (Line $40F_5$ x 'Waimanalo'-23) and $317F_2$

Table 7. Percent defoliation obtained one month after inoculation in F_2 papaya population of seedlings inoculated at one month of age

Lines	% defoliation	
	Transformed[z]	Original
	arc sine $\sqrt{\%}$	%
'Higgins' (Control, susceptible line)	51.72a	61.66
$315F_2$ (Line $40F_5$ x Line $45F_6T_{22}$)	50.92ab	60.18
$341F_2$ (Line $40F_5$ x 'Higgins')	46.41abc	52.47
$318AF_2$ ('Waimanalo'-23 x 'Higgins')	45.73bc	51.32
$319F_2$ (Line $45F_6T_{22}$ x 'Higgins')	45.09c	50.18
$318BF_2$ ('Waimanalo'-24 x 'Higgins')	43.74cd	47.91
$316AF_2$ ('Waimanalo'-23 x Line $45F_6T_{22}$)	41.62cd	44.16
$316BF_2$ (Line $45F_6T_{22}$ x 'Waimanalo'-24)	38.42de	38.83
$314AF_2$ (Line $40F_5$ x 'Waimanalo'-23)	35.88ef	34.43
$317F_2$ ('Waimanalo'-23 x 'Waimanalo'-24)	34.52ef	32.58
$314BF_2$ (Line $40F_5$ x 'Waimanalo'-24)	32.62f	29.74

CV = 11.00%

[z]Mean separation by BLSD test, 5% level

('Waimanalo'-23 x 'Waimanalo'-24) with 34.43 and 32.58 percent, respectively.

Height of one month old seedlings was measured prior to inoculation and again one month after inoculation (Table 8). Initial height differences among the populations may be attributed to their genotypic expressions.

Differences in height obtained during the one month period after inoculation were significant at the 5% level, but the possibility exists that the differences in height may also be due to their genotypic expression and not to root rot resistance. Line $316AF_2$, $319F_2$, $316BF_2$, $315F_2$ and $314BF_2$ were affected by the disease (Table 8). This is in agreement with previous studies (Mosqueda-Vazquez, 1977) that when 'Higgins' or Line $45F_6T_{22}$ was involved as a parent, growth rate and plant height were reduced. The growth rate of the susceptible Line, 'Higgins', was unexpectedly high. The 10.78 cm. growth fell within the statistical range of the more tolerant populations. The height growth rates during the inoculated period do not seem to be related to percent defoliation in several cases. 'Higgins' showed the highest percent defoliation (Table 7), but height increase was comparable to some of the more tolerant lines. Line $318AF_2$ showed 51.32 defoliation (Table 7), but showed an intermediate height increase of 11.49 cm. The lowest increase in height was shown by $314BF_2$ (Line $40F_5$ x 'Waimanalo'-24) with 6.77 cm. This is also unexpected

Table 8. Mean height of one month old F_2 papaya population measured prior to the inoculation and one month after inoculation

Lines	Mean height in cm.[z] Prior inoc.	1 mo. after	Difference
$317F_2$ ('Waimanalo'-23 x 'Waimanalo'-24)	8.57a	22.79a	14.24a
$315F_2$ (Line $40F_5$ x $45F_6T_{22}$)	7.27b	14.68de	7.79e
'Higgins' (Control, susceptible line)	7.01bc	18.05bc	10.78bc
$316AF_2$ ('Waimanalo'-23 x Line $45F_6T_{22}$)	6.76bc	15.25cde	8.55de
$314AF_2$ (Line $40F_5$ x 'Waimanalo'-23)	6.46bcd	19.16ab	12.58ab
$319F_2$ (Line $45F_6T_{22}$ x 'Higgins')	6.32bcd	14.46def	8.24de
$318BF_2$ ('Waimanalo'-24 x 'Higgins')	6.31bcd	17.97bc	11.67bc
$318AF_2$ ('Waimanalo'-23 x 'Higgins')	6.21bcd	17.06cd	11.49bc
$341F_2$ (Line $40F_5$ x 'Higgins')	5.77cde	15.98cde	10.12cd
$316BF_2$ (Line $45F_6T_{22}$ x 'Waimanalo'-24)	5.23de	13.47ef	8.08de
$314BF_2$ (Line $40F_5$ x 'Waimanalo'-24)	4.94e	11.69f	6.77e
CV %	15.84	14.95	22.86

[z]Mean separation by BLSD test, 5% level

inasmuch as Line $314AF_2$, a similar cross having a different plant selection of 'Waimanalo' as a parent, showed high growth response. The parental lines, Line $40F_5$ and 'Waimanalo'-24 were classified as tolerant by Mosqueda-Vazquez (1977). This may be explained by the fact that the rate growth of $314BF_2$ was slow even before inoculation (Table 8).

SUMMARY AND CONCLUSIONS

Ten F_2 populations derived from F_1's used in a previous half diallel study were used in this research. The objectives were to study the percent mortality, vigor rating and percent selectable plants after greenhouse inoculation, using an inoculum level of 3800 sporangia of *P. palmivora* per plant. Data for these parameters were taken one month after inoculation. Assessment of growth reduction caused by the disease was done by utilizing data on percent defoliation and plant height increases.

Results obtained from the 10 F_2 lines and the control line 'Higgins' showed higher mortality than those obtained in the F_1's by Mosqueda-Vazquez (1977) because a different isolate and a higher concentration of sporangia per plant was used. The infection intensity was increased to a level in which a relatively skewed distribution was produced.

Results obtained in plant mortality in these F_2 populations are consistent with the presence of resistance to papaya root rot among groups involving parents classified as resistant (Mosqueda-Vazquez, 1977). There was generally a high level of resistance in the intermediate resistant and susceptible groups when crossed with resistant parents. However, some evidence of variations in combining ability were shown among the F_2 populations involving specific parents. 'Waimanalo'-24 as a parent line gave better resistance to *P. palmivora* root rot in crosses with 'Higgins' and Line $40F_5$ than 'Waimanalo'-23. On the other hand, 'Waimanalo'-23 gave better resistance to Line $45F_6T_{22}$ than 'Waimanalo'-24 and Line $40F_5$ as resistant parents.

Among the three parameters used to evaluate resistance, vigor ratings of seedlings done one month after inoculation were found to be more useful than just percent mortality as it also provided the frequency distributions of the individuals within the population, although percent mortality gave slightly better mean separation for the F_2 populations.

Vigor ratings varied from 1.30 in the F_2 population of Line $45F_6T_{22}$ x 'Higgins' to 2.84 in the F_2 populations of 'Waimanalo'-23 x 'Waimanalo'-24 in a rating scale of 1 to 5 (1 = dead; 5 = healthy). However, there appeared to be some evidences of nonallelic interactions with genes of Line $45F_6T_{22}$ suppressing the genes for resistance from Line $40F_5$ interaction which was also suggested by Mosqueda-Vazquez (1977).

Frequency distribution histograms are presented to show the partitioning of the seedlings into the 5 vigor rating classes for the 10 F_2 populations and the susceptible inbred used as control. The interactions mentiond earlier may be illustrated by these histograms. $314BF_2$ (Line $40F_5$ x 'Waimanalo'-24) showed an increase in the number of plants in the 5 vigor rating class as compared fo Line $314AF_2$ (Line $40F_5$ x 'Waimanalo'-23).

Variation found in the 10 F_2 populations was higher than the variation found in the homozygous line 'Higgins', suggesting that segregation to root rot resistance was present in the F_2 populations studied.

Selectable plants are the plants that are vigorous enough to be accepted in commercial fields. These plants are the ones rated 4 or higher. As expected, 'Higgins' and those F_2 populations in which it was involved as a parent produced the lowest percentages of selectable plants. On the other hand, the sib-mated 'Waimanalo' and Line $40F_5$ times the two 'Waimanalo' sublines gave the highest percentage of selectable plant.

Foliar defoliation data seem to be in agreement with Mosqueda-Vazquez (1977). In this study the F_2 population of Line $40F_5$ x Line $45F_6T_{22}$ and the control showed the most severe defoliation. The lowest defoliation was in $314BF_2$ (Line $40F_5$ x 'Waimanalo'-24) which originated from two resistant parents.

Height of the papaya seedlings presented different responses. It appears that this factor has no direct correlation with root rot resistance. Initial measurements before inoculation showed differences in height which may be attributed to genotypic expressions. Differences in growth obtained during the one month period after inoculation were significantly different at the 5% level. Line $316AF_2$, $319F_2$, $316BF_2$, $315F_2$ and $314BF_2$ were adversely affected. This is in agreement with previous studies

(Mosqueda-Vazquez, 1977) that showed reduced plant growth when 'Higgins' or Line $45F_6T_{22}$ was involved as a parent. However, 'Higgins' used as a susceptible control line exhibited an unexpected high growth rate. The smallest increase in height was manifested by $314BF_2$ (Line $40F_5$ x 'Waimanalo'-24). This was also unexpected inasmuch as both parents are considered to be resistant.

The 10 F_2 populations are listed here in 3 groups according to their reactions to the pathogenic organism: Resistant group: $317F_2$, $314BF_2$ and $314AF_2$; Intermediate group: $318BF_2$, $316AF_2$, $341F_2$, $316BF_2$, $315F_2$ and $318AF_2$; Susceptible group: $319F_2$.

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