GENETIC AND CULTURAL CONTROL OF ANTHURIUM BURROWING NEMATODE, *RADOPHOLUS CITROPHILUS*

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Koon-Hui Wang

Thesis Committee:
Adelheid Kuehnle, Chairperson
Brent Sipes
Hector Valenzuela

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

Adelheid R. Kuehle
Chairperson

Brent Sipes

Hector Valverde

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Abstract

Cinder medium is able to decrease burrowing nematode damage in 'Alii'. This provides a supplemental method to manage anthurium decline on burrowing nematode intolerant cultivars. Burrowing nematode can invade *Anthurium* shoot tissues, thus propagation by shoot top cutting cannot guarantee nematode-free planting materials. In vitro screening for burrowing nematode tolerant and resistant cultivars identified 'Midori' as standard tolerant and 'Nitta' as standard susceptible cultivars. Future breeding could select progenies that are more tolerant than 'Midori' and moderately low in nematode parasitism index [log (Rf+1) of progeny / log(Rf+1) of 'Nitta'], where Rf is final nematode number / initial nematode number. Nonparametric parameters and parameters adjusted to initial reading and uninoculated reading are effective in evaluating burrowing nematode tolerance. Based on in vitro screening, *Anthurium pittieri* and *A. ravenii* were evaluated as tolerant species. *A. ravenii*, *A. aripoense* and *A. pittieri* were evaluated as resistant species. (Ca+Mg)/K is negatively correlated with burrowing nematode tolerance and resistance in *Anthurium* and could provide a good marker for nematode resistance and tolerance selection.

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Chapter 1. Literature Review

1.1. Anthurium Decline

Burrowing nematode (BN) infection is a serious and persistent problem affecting *Anthurium* production in Hawaii. Surveys of 10 *Anthurium* farms on the island of Hawaii (representing approximately half of total *Anthurium* production in acreage Hawaii) revealed burrowing nematodes in all samples (Goo, 1995; K. Sewake, UH Hilo cooperate Extention Service, personal communication). Decreased flower yield in *Anthurium* due to burrowing nematode infection can reach up to 50% (Aragaki and Apt, 1984). Infected plants become stunted, the root system is destroyed, and subsequently the roots rot. Together these symptoms are commonly known as anthurium decline.

New plantings propagated from shoot (top) cuttings are vigorous and may be productive for 2 to 3 years in an infested field. However, plants propagated from rooted basal stem cuttings usually express decline symptoms sooner than those from shoot cuttings. Diseased plants in a field usually are clustered, and are often associated with poor field drainage (Aragaki and Apt, 1984). Plant damage is enhanced by secondary infection and results in rotten roots. The fungi, *Pythium ultimum* Trow, *P. splendens* Braun, other *Pythium* spp., *Rhizoctonia* sp. (binucleate), *Phytophtohora cinnamomi* Rands and *Calonectria crotalariae* (Loos) Bell & Sobers have been isolated from nematode infected roots (Aragaki and Apt, 1984).

The sources of BN contamination could be diseased cuttings, insufficiently fumigated old substrate and soil, contaminated water, diseased culture medium, and could arise through inappropriate hygienic practices including use of contaminated footwear and tools (Holtzmann et al., 1984). In Hawaii, most field infections result from previously infected planting materials or contamination from plants of the families Araceae, Musaceae, and Zingiberaceae (Schenck and Holtzmann, 1990).

In addition to decreased flower yield and plant loss, a main drawback of BN infestation on *Anthurium* production in Hawaii is a quarantine restriction that bars export of infected potted *Anthurium*. Quarantine restrictions in Japan (L. Wong, personal communication) and California (Holdeman, 1986b) prohibit the entry of all BN contaminated potted plants. A Japan-Hawaii Burrowing Nematode Certification Program was developed that allows export of potted plants to Japan after obtaining a Federal Phytosanitary Certificate in accordance with Chapter 73 of Plant Export Rules, Hawaii Administrative Rules.

1.2. Burrowing Nematode, *Radopholus similis,* sensu lato

Burrowing nematode, *Radopholus similis* (Cobb, 1893), Thorne, 1949, *sensu lato* (the species in the broad sense), phylum Nemata, is a migratory plant endoparasitic nematode. The mature female varies in length from 0.7 to 0.9 mm and in diameter from 0.020 to 0.024 mm. The life cycle of BN varies from 18 to 22 days. This encompasses development from the egg and passage through four juvenile stages, to the adult males or females, plus another 2 days to begin laying eggs. Though bisexual reproduction is normal for BN, parthenogenic reproduction is also possible (Huettel and Dickson, 1981).

Invasion

According to DuCharme's (1959) study on BN invasion in citrus roots, BN can invade any succulent plant part or succulent tissue touching the soil, but favors invasion of the area near the root tip. The nematode penetrates the epidermis and tunnels into the cortex by dissolving the cells. The penetration stops at the fibrous central stele and suberized endodermis. Other tissues that cannot be attacked are suberized exodermis, mature xylem, hardened pith periderm, and hardened root cap. Death of invaded tissue forces the nematode to search for new feeding sites (DuCharme, 1959). Secondary infection following the BN primary infection often results in synergistic damage to its host. This

synergism (i.e., the action of two or more organisms to achieve an effect of which each is individually incapable) enables BN to penetrate the endodermis (Holdeman, 1986a).

Environmental Influences

BN is an obligate parasite unable to survive adversity (Holdeman, 1986a). Without a host, it starves to death in less than 5 months (Birchfield, 1957). Environmental factors that influence survival are soil moisture, temperature, soil texture and structure. DuCharme demonstrated that water movement through the soil is a factor affecting the migration of BN (Holdeman, 1986a). Under field conditions, BN survived only 1 month in dry soil (29-34 °C) but 6 months in moist soil (27-37 °C; Koshy and Geetha, 1993). Koshy and Geetha also found that BN could not survive moisture levels lower than 2% during all stages of its life cycle. A soil temperature of 24 °C is optimum for the citrus BN; temperatures higher than 32 °C or lower than 13 °C are lethal (Holdeman, 1986a). In general, in a temperate region, the severity of BN damage is lower in heavier soils (i.e., clays), and higher in lighter soils (i.e., sands; Holdeman, 1986a). This is related to the freedom with which the nematodes move through the soil. However, in Puerto Rico, the highest BN populations were found on the heavy clay soils.

Host Range

BN is reported to attack more than 250 species of plants worldwide. Citrus and banana are among the most important hosts affected. BN causes spreading decline on citrus in Florida (Holdeman, 1986b), black head or toppling disease in banana (Loos and Loos, 1960), and slow wilt and yellows of black pepper (Van der Vecht, 1950).

Geographic Distribution

BN is naturally distributed worldwide in the tropics and subtropics except Jordan valley, Israel and Taiwan (Koshy et al., 1991). This distribution also extends into temperate zones including 29° 30' latitude in Florida, New South Wales, Australia, and into greenhouses at higher latitudes. However, the 5-chromosome populations of BN have

only been reported on citrus in Florida, on *Anthurium* in Hawaii (Huettel et al., 1986) and on plantain or cooking banana in Puerto Rico.

Scientific Name of BN

The BN was first found by N.A. Cobb in Australia on banana material shipped from Fijii and was described and named in 1893 as *Tylenchus similis* Cobb, 1893. In 1949, G. Thorne renamed it as *Radopholus similis*. In 1956, DuCharme and Birchfield reported a difference in the ability of two Florida BN populations to colonize both citrus and banana. The BN population from citrus (CBN) was able to colonize both citrus and banana while the BN population from banana colonized banana but not citrus. They classified these populations as two "physiological races" named the "citrus race" and the "banana race". Huettel, Kaplan, and Dickson (1984) proposed that the two populations were sibling species and named the 5-chromosome BN population found in citrus in Florida and *Anthurium* in Hawaii as *R. citrophilus* Huettel, Dickson, and Kaplan, 1984. Sibling species are morphologically indistinguishable but sexually isolated. Later, in Great Britain, Siddiqui (Holdeman, 1986a) reduced the species names to subspecific level, thus *Radopholus similis similis* and *Radopholus similis citrophilus*. However, regulatory agencies disregarded the proposed sibling species concept and maintained the taxonomic status of these nematodes as races of *R. similis* sensu lato (Holdeman, 1986a).

The diagnostic characteristics between the two populations presented were:

> (1) Karyotype, *R. citrophilus* has n=5; *R. similis* has n=4 (Huettel et al., 1984a).
> (2) Diagnostic proteins. Seven enzyme-encoding loci as determined by starch gel electrophoresis were indicated as diagnostic; a major protein difference was found when comparing non-enzymatic proteins using polyacylamide slab gel (Huettel et al., 1984b).
> (3) Behavioral comparisons. Males of *R. similis* are attracted to but do not copulate with females of *R. citrophilus*, whereas *R. citrophilus* males do not respond to the banana race females at all (Huettel et al., 1982).

More recently, minor morphological differences in the tail region in males between *R. similis* and *R. citrophilus* were identified (Huettel and Yaegashi, 1988). *R. citrophilus*

has 3 to 7 genital papillae (anterior hypotymata) whereas *R. similis* had either smooth or had one or two shorter papillae. Slight differences in developmental stages of oogenesis between the two species were observed (Huettel and Dickson, 1981).

However, random amplified polymorphic DNA (RAPD) analysis suggested that the genome organization of the burrowing nematode sibling species *R. citrophilus* and *R. similis* is highly conserved (Hahn et al., 1994; Kaplan et al., 1996). Interspecific copulation between these two sibling species was also observed (D.T. Kaplan, unpublished data).

BN populations from *Anthurium* in Hawaii were identified to have five chromosomes (n=5), an isozyme pattern identical to that of *R. citrophilus,* and an ability to mate with the Florida population of *R. citrophilus*. However, BN populations from *Anthurium* in Hawaii could not persist in roots of citrus. Moreover, *Anthurium* in Hawaii was found to be a poor host of *R. similis*. Huettel, Kaplan, and Dickson (1984) concluded that BN population from Hawaii is *R. citrophilus*.

1.3. Control of Plant Parasitic Nematode

Integrated Pest Management is a critical component of sustainable agriculture. This management approach may include chemical controls, sanitation, crop rotations, the use of cover crops, soil amendments, biological control, physical control, and planting resistant or tolerant hosts.

Chemical Control

Chemical control has been the most commonly used method in controlling plant-parasitic nematodes. One of the most widely used chemicals, methyl bromide, has been used to control nematode damage by reducing preplant nematode population densities. However, federal law has frozen methyl bromide production and imports at 1991 levels and may ban all production and imports by January 1, 2001 (Beyets, 1996). Other

nematicides, like fenamiphos, are also commonly used in burrowing nematode control in *Anthurium*. Though Nemacur (fenamiphos) might not be banned within the near future, the effective application rate has already been reduced by a third as a result of a formulation change to 10G from 15G. In general, the impact of chemical control on the environment is socially unacceptable. Nonchemical methods to control burrowing nematodes are highly desirable.

Sanitation

Other recommended cultural methods to control burrowing nematode in Hawaii include preplant methods including drying of the growing media, planting only nematode-free stock, practicing 2-month fallow period every 5 years, with turning and drying of the planting beds will destroy up to 90% of the nematode population (Hara et al., 1995).

In Florida, control of BN in citrus has been with the "push and treat" method (O'Bannon, 1977). With this approach, infected trees are eradicated, the field is treated with 1,3-dichloropropane-1,2-dichloropropene (D-D) applied at a rate of 672 kg/ha, and the ground is left fallow for 2 years following treatment. Herbicides are used to maintain weed-free conditions.

Quarantine

Since *R. citrophilus* that attack citrus have only been detected in Florida, federal and state quarantines play a role to prevent the dissemination of *R. citrophilus* to other citrus production areas. BN from *Anthurium* in Hawaii was identified to be *R. citrophilus* by Huettel et al. (1984), therefore quarantine investigation on *Anthurium* is critical to control the spread of *R. citrophilus*.

A Japan-Hawaii Burrowing Nematode Certification Program was developed which allows export of potted plants to Japan after obtaining a Federal Phytosanitary Certificate in accordance with Chapter 73 of the Plant Export Rules, Hawaii Administrative Rules. Plants are not allowed to be shipped in soil and those plants that are found infested with

nematodes or other organisms are promptly isolated or removed from the certified areas. Among the soil-free and potting media approved for export are peat, sphagnum, bark charcoal, perlite, vermiculite, rockwool, pumice, and volcanic cinder. To meet the Japan-Hawaiian Burrowing Nematode Certification Program, potted plants must be grown in pest-free potting media approved by Japan Ministry of Agriculture, Forest and Fisheries (MAFF), benches or beds must meet minimum height of 45 cm with no overhanging branches over shade houses. Walkways must be concrete, volcanic cinder, crushed rock, covered in saran, or plastic, and free of soil, debris, moss and weeds. Benches and ground cover must be treated with sanitizing solution every 6 months or whenever a new planting takes place. Prevention of drainage of water from one plant to another must be considered. An assembly area for the out shipment of plants should be free of soil and debris (L. Wong, Hawaiian State Agriculture Department, personal communication).

The cultivation of *Anthurium* in an environment free of nematodes, combined with pathogen exclusion practices, would offer one solution to the threat from nematodes. The availability of sterile soilless media makes this partially possible and also meets quarantine requirements.

Crop Rotation

Rotation between resistant and tolerant cultivars is one strategy to improve nematode control. Continuous cropping of the soybean cyst nematode (SCN) resistant soybean 'Bedford' resulted in increased reproduction of the SCN population as compared to rotation of resistant and susceptible cultivars (Young and Hartwig, 1992).

Crop rotation with non-hosts is not suitable for *Anthurium* culture as the crop is usually replanted every 5 years or longer (Higaki and Poole, 1978; Higaki et al., 1994).

Soil Amendment

Soil amendments with organic compost and fertilization were found to be effective in controlling some nematodes. Application of nitrogen decreased plant damage symptoms

in tea caused by *Pratylenchus loosi* and in cereals by *Heterodera* (Brown, 1987). Association with vesicular-arbuscular mycorrhizal fungi increased rough lemon species tolerance to *R. similis* due to increase absorption of P (Smith and Kaplan, 1988). Leaf extracts from *Glyricidia maculata, Ricinus communis*, and *Crotolaria juncea*, and chopped leaves of *G. maculata* as green manure were lethal to *R. similis* (Jasy and Koshy, 1992). *R. similis* also can be controlled by leaf extracts of *Tagetes patula* (Subramaniyan, 1985).

Biological Control

The fungus, *Paecilomyces lilacinus*, reduced the damaging effects of *R. similis* during nematode inoculation on betel vine (Sosamma et al., 1994). In a survey of antagonists of nematodes in citrus and noncitrus groves in Florida, approximately 24 species of microbial antagonists capable of attacking vermiform stages of *R. citrophilus* were recovered.*Verticilium chlamydosporium* Goddard was isolated from vermiform burrowing nematodes. An actinomycete, *Streptomyces* sp., was recovered from dead burrowing nematodes; sixteen mites, and a springtail, *Onychiurus* sp. readily fed on burrowing nematode in the laboratory (Walter and Kaplan, 1990).

Hot Water Treatment

Hot water treatment is used to control burrowing nematode in *Anthurium* (Higaki and Poole, 1978). According to Kaplan, 49 °C for 10 minutes should kill nematodes, with more time needed for very woody materials (A. H. Hara, UH Department of Entomology, personal communication).

Since sources of nematode contamination are difficult to determine and treatments such as soil amendment, biological control or physical control have not eradicated nematodes, plant genetic tolerance and resistance to nematode should be considered. In fact, there is evidence that resistance offers more effective control of *Globodera rostochiensis* and *G. pallida* on potato than chemical treatment (Trudgill et al., 1978; Gurr, 1992).

1.4. Growing Medium

Anthurium Growing Medium

Anthurium grows best in a medium that is well-aerated and has good water holding capacity. Volcanic cinder is the most commonly used medium for *Anthurium* culture in Hawaii because of its availability and low cost. Organic media like peat moss are often mixed with cinder to increase water holding capacity. Other media used over the years in Hawaiian *Anthurium* production include woodshavings, sugarcane bagasse, hapuu chips, taro peel, macadamia nut shells, coffee parchment, rockwool, and polyfenol foam (Higaki and Poole, 1978).

Interaction of Nematodes with Plant Growth Medium

Soil properties, mainly soil texture, moisture, and pH can greatly influence the population behavior of the citrus nematode, *Tylenchulus semipenetrans,* on citrus (O'Bannon and Ford, 1976; O'Bannon, 1967; Van Gundy, 1964). CBN migrated a longer distance and reproduced best in well-drained soil (Holdeman, 1986a; O'Bannon and Ford, 1976). Van Gundy related this phenomenon to soil nutrient content where increasing amounts of sand sharply decreased root K, and increased root Ca, in citrus seedlings grown in an alkaline soil (1964). Nematode infection may also be related to organic matter content in the growing media. Organic matter enhances nematode infection, causing early damage, but is unfavorable for ultimate high densities of citrus nematodes (O'Bannon, 1967). Peatmoss may have created a thin coat over the citrus roots, encouraging infection by the nematode (Van Gundy et al., 1964). Stunting of citrus seedlings by citrus nematode was greater in wetter soils than in drier soils and greater in pH 7.5 than in pH 6.0 (Van Gundy et al., 1964). Soil particle size is another factor affecting nematode reproduction. Coarse textured soils favor CBN damage to citrus (O'Bannon and Ford, 1976). The size of the sand, including the percentages of very fine and fine sand will affect the activity of CBN (Holdeman, 1986a,b). The greatest CBN damage to a citrus tree occurs in deep,

well-drained sands with 5 to 7% moisture-holding capacity and a permanent wilting point of approximately 2.5%. CBN reproductive potential and activities are highest in subsoils containing greater than 95% sand and less than 0.25% organic matter.

1.5. Genetic Control of Nematodes

Definition of Resistance and Tolerance

The concepts of 'resistance' and 'tolerance' by nematologists are different from those for other pathogens (bacteria, virus, and fungi). In other branches of plant pathology, the number of the pathogens in the infected plants is too much or too small to count, therefore disease symptoms are used as an assessment of resistance. Trudgill recognized these differences but accepted that 'resistance' is used throughout plant pathology to described the ability of the host to resist or hinder pathogen invasion, development or multiplication; 'tolerance' describes the extent to which the host is able to withstand infection without suffering undue damage. In nematology, resistance may be independent of tolerance (Trudgill, 1985). The extremes of resistance and tolerance of nematodes in the host may be represented in a four-celled chart (Table 1.1).

Table 1.1. Extremes of nematode resistance and tolerance according to Trudgill (1985).

		Host Growth	
		Good	Poor
Pathogen Reproduction	Good	Tolerant Susceptible	Intolerant Susceptible
	Poor	Tolerant Resistant	Intolerant Resistant

Genetic Studies of Resistance and Tolerance

The type of feeding by nematodes influences the potential availability of resistance genes (Boerma and Hussey, 1992). Ectoparasitic nematodes that remain outside of the plant tissue and attack the plant epidermal cells with their stylet do not establish a lasting relationship with their host and therefore are unlikely to exert selection pressure on the host for the evolution of resistance genes. Migratory endoparasitic nematodes enter and migrate within plant tissues but do not require a specialized host response for successful parasitism. Therefore, antagonistic host responses that suppress nematode development and reproduction have been identified in a limited number of crops for this nematode. Burrowing nematode belongs to this later group; limited examples of host resistance have been found against this nematode. Sedentary endoparasitic nematodes maintain a highly specialized feeding relationship with their hosts that has resulted in the evolution of resistance genes in many crop species. In general, as nematode parasitism becomes specialized with a concomitant restriction in host range, the potential for identifying resistant genes greatly increases.

A review of resistance to plant-parasitic nematodes found that 52% are monogenic, 28% are oligogenic and 20% are polygenic (Boerma and Hussey, 1992). Since burrowing nematode is a migratory endoparasitic nematode, host resistance to this nematode might be limited. However, some burrowing nematode resistant rootstocks have been identified in Milam lemon (*Citrus limon* × 'Milam'), Ridge Pineapple (*Citrus sinensis*), and Carrizo citrange (*Poncirus trifoliata* × *Citrus sinensis*) (Kaplan, 1990).

Nevertheless, use of nematode-resistant plants has a potential disadvantage. It may result in selection against the host resistance gene and in selection for resistance-breaking nematodes (reviewed by Roberts, 1992). Nematode populations are believed to be heterogeneous and often differ in the ability to reproduce on hosts carrying genes for resistance (Young and Hartwig, 1992). Evidence suggests a gene-for-gene relationship

between resistance gene H1 from *Solanum tuberosum* ssp. *andigena* and a gene in *Globodera rostochiensis*, and betweeen H2 from *S. multidissectum* and a gene in *G. pallida* (Parrott, 1981). These virulence genes (virulence is defined by Vanderplank as the ability in a nematode to reproduce on a plant with one or more major resistance genes; Trudgill, 1985) were homozygous single recessive genes (Parrott, 1981).

However, Parliet and Zadoks proposed a gene-for-gene theory that also applied to race non-specific resistance or a polygenic resistance system (Turner et al., 1983; Roberts, 1992). A resistance-breaking potato cyst nematode pathotype was also found by Turner and Perry in an *S. vernei* hybrid (Turner et al., 1983). *S. vernei* is a potato cyst nematode race non-specific resistant cultivar. Within five generations of virulence selection on this *S. vernei* pathotype, 80% susceptibility of *S. vernei* to potato cyst nematode was found. Resistance breaking emphasizes the disadvantage of using host plant resistance as a control strategy. The only acceptable BN-resistant citrus root-stock, 'Milam' was infected by a new biotype of the citrus pathotype of BN (Kaplan and O'Bannon, 1985). Examples of some successful nematode resistance are listed in Table 1.2.

Table 1.2. Examples of some nematode resistant genes in plants (reviewed by Fassuliotis, 1987).

Host	Nematode	Source	Gene
potato	*Globodera rostochiensis*	*Solanum tuberosum* ssp. *andigena*	H1 (only race Ro1 and Ro4)
potato	*G. pallida*	*S. vernei*	polygenic
potato	*G. pallida*	*S. gourlayi*	unknown
potato	*Meloidogyne incognita*	*S. sparsipalum*	2 dominant genes
potato	*M. javanica*	*S. sparsipalum*	3 dominant genes

Table 1.2 (Continued)

Host	Nematode	Source	Gene
potato	*M. arenaria* .	*S. sparsipalum*	> 3 dominant genes
potato	*M. hapla*	*Solanum tuberosum* ssp. *andigena*	unknown
tomato	*M.* spp.	*Lycopersicum peruvianum*	single dominant or incomplete dominant
tomato	*M. incognita*	'cold set'	recessive gene
tomato	*M. incognita*	*L. peruvianum*	Mi, single dominant gene
tomato	*M. hapla*	*L. peruvianum*	unknown
tomato	*M. hapla*	*L. glandulosum*	unknown
tomato	*Rotylenchus reniformis*	*L. pimpinellifolium*	monogenic
pepper	*M. incognita* *M. javanica* *M. arenaria*	*Capsicum* spp	1-4 dominant genes
eggplant	*M. incognita* *M. javanica*	*Solanum sisymbriifolium*	unknown
tobacco	*M. cognita*	*Nicotiana tomentosiformis*	single dominant
	M. spp.	Sweet potato	quantitative
cotton	*R. reniformis*	*Gossypium stocksii G. somalense G.barbadense* 'Texas 110'	quantitative, 2 or 3 pairs of recessive genes
alfalfa	*Ditylenchus dipsaci,* *M. hapla,* *M. javanica*	vernal alfalfa	single dominant, tetrasomic genes
cowpea	*M. incognita,* *M. javanica* *M. hapla*	natural resistance	single dominant

Studies on resistance gene relationships with races of soybean cyst nematode (SCN) showed the complication of resistant genes. The resistant genes varied among

different sources of resistant hosts and introductions to races of SCN (Myers and Anand, 1991).

The expression of resistance to nematodes can be modified by environmental conditions that affect defense mechanisms in many crop species. These factors include temperature, nutritional status, soil pH, and plant age (Canals and Pinochet, 1992). For example, peach-almond hybrids infected with *M. javanica* expressed resistance in 1-year-old plants but not on 1-month-old plants, and lost some partial resistance with an increase in temperature (Canals and Pinochet, 1992).

Studies in the tolerance of plants to nematode attack became significant in the 1970s (Evans and Haydock, 1990). Nematode tolerance was found to be quantitatively inherited. For example, tolerance of soybean to SCN had a broad sense heritability of 31% in subplots untreated with nematicide (Reese et al., 1988). Since tolerance is basically a measurement of plant yield, the genetics of tolerance are expected to be complicated. Genetics of tolerance have been studied less than resistance. However, since, nematode tolerance is apparently race-independent (Reese et al., 1988), selection for tolerance would be expected to offer longer-term plant protection than selection for resistance.

Mechanisms of Nematode Resistance

The mechanism of host plant resistance may be active or passive. Active, or post-infectional resistance is the response of the plant after infection by a parasite. Subsequent phytoalexin formation, which can inhibit nematode mobility, suggests this to be a hypersensitive response (Cooks and Evans, 1987). Other substances might also be produced in plants in response to nematode infection. For example, as reviewed by Cook and Evans (1987), soybean roots produce glyceolin after infection by *M. incognita*, and cotton produces terpenoids after infection by *M. incognita*. Such responses have temporal and spatial differences. As the nematode surface is dynamic, phytoalexins might

immobilize the juvenile stage of *M. incognita* but have no effect on the adult stage (Reddigari et al., 1986).

Passive, or preinfectional, resistance is related to the physiological, physical, and chemical properties of the plant (Cook and Evans, 1987). This includes thickened tissues as a limiting factor for penetration, toxins that kill nematodes, or inadequate food supply for the nematodes (Cook and Evans, 1987).

Development of an effective nematode resistant cultivar requires an understanding of the mechanism of plant compatibility with nematodes and of the genetic basis of plant resistance to nematodes. The mechanism of plant and nematode compatibility has been studied. Nonhost-specific plant nematodes are thought to recognize thermal variation and carbon dioxide levels in the soil. In contrast, host-specific nematode infection is due to chemotaxis (Zuckerman and Jansson, 1984). Molecular evidence shows that the chemical recognition is caused by a lectin-carbohydrate interaction, i.e., the carbohydrate on the nematode surface acts as a receptor to the lectin on plant tissues (Kaplan and Davis, 1987; Kaplan and Gottwald, 1992; Zuckerman and Jansson, 1984).

The plant genetic mechanisms controlling formation of the nematode feeding site are mostly studied in sedentary endoparasitic nematodes. The response of potatoes carrying the H1 gene confering resistance to *G. rostochiensis* is to prevent the expansion of the feeding site to the transfer-cell state, upon which development of the female depends. (reviewed by Opperman et al., 1994). The resistance mechanism in cowpea is due to the presence of a cowpea trypsin inhibitor. This inhibitor reduces the fecundity of *Meloidogyne* spp. and influences the sexual fate of recently established *G. pallida*, biasing the population towards excessive production of the much smaller and less damaging males (reviewed by Opperman et al., 1994).

Mechanism of Nematode Tolerance

The most commonly used measurement for tolerance in the field is plant yield from treated plots as a portion of the yield from untreated plots (tolerance index). This is described in detail in the next section. Many other plant responses are measured to look at their correlation with the tolerance index. This has lead to an increased understanding of the tolerance mechanisms. These are discussed below.

> (1) Water use efficiency. The more heavily infected plants had greater stomatal resistance and more negative leaf water potentials (in Evans and Haydock, 1990). Klar and Franco also found that nematode-tolerant cultivars were more drought tolerant (in Evans and Haydock, 1990). However, Volkmar (1990) studying the infection of oats by cereal cyst nematode, *Heterodera avenae*, reported that water use efficiency was not correlated with the tolerance index.
> (2) Root system efficiency. An observed variation in tolerance to nematode infection was attributed to differences in the total root weight of infected plants (Evans, 1982). Though some research suggested that the shoot to root weight ratio could also be used as a measurement for tolerance, Evans (1982), and Trudgill and Cotes (1983) disproved this. Miltner et al. (1991) suggested that compensatory growth and the possible greater efficiency of root system of the SCN tolerant soybean cultivar, 'Wright', may have contributed to its tolerance. In fact the number of growing roots of 'Wright' was stimulated by the presence of SCN.

In addition, plant nutrition is a major factor which might contribute to plant nematode tolerance. This will be discussed section 1.7.

Screening Methods

Most of the current screening for nematode resistance is done in the field. However, some disadvantages to field screening are: (1) nematode infestation in the field is not uniform; (2) restriction on nematode reproduction during winter; (3) a polyspecific nematode community is present and becomes a complication (Boerma and Hussey, 1992).

In contrast, in vitro screening offers some advantages to overcome these problems. With the application of in vitro screening, inoculum level is controllable and uniform. In addition, the inoculum is composed of non-indigenous nematode species or races at the place of evaluation, thus the tested plants may not have previously gained adaptation to this

inoculum. In vitro screening is also free of seasonal effects during evaluation (Boerma and Hussey, 1992).

One question raised by in vitro screening is whether or not the results are applicable to plants growing ex vitro. Therefore, repeating the screening procedure in the greenhouse or field environment is necessary to test the consistency between the in vitro and ex vitro results. Screening self-rooted peach cultivars and rootstocks for their response in vitro to *M. incognita* using hormone-free medium showed correlation with at least one parameter measured under field conditions (Huettel and Hammerschlag, 1993). However growth regulatory substances, for example cytokinin, appeared to result in an initial loss of resistance to *M. incognita* during in vitro screening of peaches (Huettel, 1986).

Assessment of Nematode Tolerance

According to Trudgill (1985), assessment of nematode tolerance is based on plant yield. Different approaches to measure plant yield exit. The common method is to measure plant growth. For example, Evans and Franco (1979) used the area of ground covered with potato leaves as a measurement for plant growth in screening for potato cyst nematode tolerance. Anand and Koenning (1986) used a yield response index, i.e., (yield without nematicide / yield with nematicide)×100. Measurement of plant damage was conducted for *R. similis* on banana using root-lesion ratings. Nematode damage was assessed by looking at the lesion size and lesion number or the percentage of surface of the banana rhizome covered with lesions (reviewed by Kaplan, 1990).

Another method to measure nematode tolerance is to compare the performance of different cultivars in plots with a range of initial nematode population densities. The slopes of regression of yield to initial nematode populations for different cultivars indicate relative tolerance. Cultivars with steeper slopes are less tolerant than those with moderate slopes (Wallace, 1987).

Assessment of Nematode Resistance

A measurement for resistance is the nematode reproduction rate. As reviewed by Trudgill (1985), the measurement widely used for potato cyst nematode is the reproductive factor, Rf (=Pf/Pi, final nematode population divided by initial nematode population). However, there are some problems with this measurement. First, the multiplication rate of cyst nematode is inconsistent because the rate decreases as the population density increases (Trudgill, 1985). Second, nematode reproduction rates vary in different environments.

A solution to these problems was developed by Phillips and Trudgill (Trudgill, 1985). Instead of looking at the Rf of a cultivar, the ranking sequence of a series of cultivars was considered to be more important in determining resistance. This is based on a study that showed that the ranking of a series of partly resistant cultivars remained consistent even when experiments were conducted in different environments (pots or field) or when there were large differences in overall nematode multiplication rates (Trudgill, 1985).

Another approach to assessing resistance is to express the number of cysts formed on partially resistant cultivars as a percentage of that on a susceptible control. Considering that susceptible cultivars can differ in their susceptibility, several cultivars should be included as controls (Trudgill, 1985).

Through nematode resistance and tolerance screening, reference standards for standard tolerant, intolerant, susceptible, or resistant cultivars can be obtained. This would be helpful in breeding programs as: (1) it can help normalize variations in test conditions; (2) standard genotypes can be used to develop a rating scale; and (3) it facilitates the identification of genotypes with superior resistance levels (Boerma and Hussey, 1992).

1.6. *Anthurium* Genetics and Breeding

Anthurium (Araceae) is a distinct neotropical genus of about 1000 species (Croat, 1988). The genus ranges from Northern Mexico and Greater Antilles to Southern Brazil and Northern Argentina and Paraguay (Croat, 1988). Engler (1905) placed 486 *Anthurium* species into 18 sections. Croat and Sheffer (Croat and Sheffer, 1983) somewhat modified Engler's system and rearranged numerous species, including the placement of *A. andraeanum,* which belongs into the section *Calomystrium* instead of section *Belolonchium.* Two of the most important species for *Anthurium* cultivation are *A. andraeanum* and *A. scherzerianum. A. andraeanum* belongs to Section *Calomystrium,* important for cut flowers in Hawaii. *A. scherzerianum,* belongs to Section *Porphyrochitonium,* and important as *Anthurium* potted plants in temperate regions.

Anthurium Reproduction

The *Anthurium* flower is hermaphroditic and protogynous (the stigma is receptive before the pollen is shed), with self-pollination within one flower are impossible. Therefore, *Anthurium* breeding is based on clonal propagation of individual selections rather than on F1 creation of hybrids from inbreds. Embryogenesis has been recently described (Matsumoto, 1994).

Genetics of *Anthurium* Spathe and Spadix Color

The only trait rigorously studied in *Anthurium* genetics is the spathe color. An overview of the inheritance of spathe color is given by Kamemoto and Kuehnle (1996). Red, pink, orange, coral, and white were classified into two groups: the red group composed of red and pink; and the orange group composed of orange and coral. Two major genes are responsible for these colors. One gene, *M*, controls the production of cyanidin 3-rutinoside, and a second gene, *O*, controls the production of pelargonidin 3-rutinoside. Expression of pelargonidin produces coral to orange spathes, whereas expression of pelargonidin and cyanidin produces pink to red. Red is dominant over

orange. Orange is homozygous and coral is heterozygous at the*O* locus; both orange and coral are double recessive at the *M* locus. White arises by the double recessive at the *O* locus with recessive epistasis over the *M* locus. Pink is heterozygous at both loci (M and O). Thus, *mmoo*, *Mmoo*, and *MMoo* produce white phenotypes, *mmOo* and *mmOO* produce orange group phenotypes, and *MmOo*, *MmOO*, *MMOo*, and *MMOO* produce red group phenotypes. Dosages of *M* and *O* affect the range of color obtained.

Other colors of *Anthurium* include purple, blush, green, brown and obake. Genetics of blush is difficult to determine because of the confounding of spath and spadix colors as well as seasonal variations in expression. Purple color is controlled by a recessive *pp* genotype, but with the presence of at least one dominant allele at the *O* locus. The purple color is probably influenced by copigmentation of cyanidin and peonidin, and pH of plant tissues. Green color depends on the presence of chlorophyll without anthocyanins. The coexistence of chlorophyll and the orange anthocyanins will result in brown color.

Spadix color was controlled by two genes, the *C* and *R* genes. Genes *C* and *R* in combination produce red to orange-red spadices, and the gene *C* produces orange to coral spadices, while the lack of both results in a nonred (yellow) spadix. The *cc* genes are epistatic to the *R* genes.

Anthurium Breeding Objectives (Kamemoto and Kuehnle, 1996; Kuehnle et al., 1996)

A) Cut flower

1. Flower yield - A minimum annual yield of six flowers per plant stem is desirable.
2. Clear, glossy and uniform spathe color; broad, symmetrical, and heart-shaped with slightly overlapping basal lobes are desired for standard *Anthurium*. Difference color intensity are preferred for obake *Anthurium*. The spathe should be angled at about 45° from the flower stem axis.
3. Spadix - Shorter than the length of the spathe and curved slightly downward is desirable.
4. Flower stem - An erect flower stem longer than the petiole is desirable.
5. Internode length - A relatively short internode to delay the need for transplanting.
6. Sucker production - Prolific sucker production was once desirable. However, with effective micropropagation procedures, sucker production has lost some importance.
7. Keeping quality - Vase life longer than 3 weeks is desirable.

8. Anthracnose resistant.
9. Bacteria blight resistant and tolerant.
10. New shapes and colors - Tulip type (cupped and upright spathes, upright spadixes). The species collected with tulip-type flowers were *A. formosum*, *A. kamemotoanum*, *A. lindenianum* and *A. nymphaeifolium*, *A. hoffmannii*, and *A. amnicola*. *A. amnicola* has contributed to the miniature tulip-type as well as to the lavender and purple spathe hybrids.

B) Potted plant

1. Foliage and plant habit - Attractive, glossy, dark green leaves; vigorous sucker production with short internodes.
2. Flowers - Flowers positioned above the foliage, attractive and contrasting colors of spathe and spadix, fragrance, novel colors, standard shapes, and numerous flowers are desirable.
3. Disease resistance - Resistance to bacterial blight, *Xanthomonas campestris* pv. *dieffenbachiae* and anthracnose or black nose, *Collectotrichum gloeosporioides*.
4. Production - Short culture period from plugs to 6-8" containers or 10" for some *A. andraeanum* types (10-14 months).
5. Post production - Tolerate shipping condition; maintain flower production indoor; retain flower color or turn greenish, without browning, with age.

These breeding objectives might have narrowed the genetic base from which to select for nematode resistance among cultivars. Thus, *Anthurium* species might provide a good source for CBN resistant or tolerant germplasm. Fehr (1987) set priorities of searching for resistance as follows: i) commercial cultivars of self-pollinators, inbred parents of hybrid cultivars, or parents of synthetic cultivars; ii) elite breeding lines that may soon become cultivars; iii) acceptable breeding lines with superiority for one or a few characters (i.e., germplasm lines or obsolete cultivars); and iv) plant introductions of the cultivated species.

Of interest here is the possibility of transferring nematode resistance or tolerance into an acceptable commercial cultivar. Besides the narrow genetics of commercial cultivars, estimation of more than half *Anthurium* production acreage in Hawaii were infested with burrowing nematode, indicates that nematode resistance among current cultivars might be infrequent. The present elite breeding lines also have many desirable characteristics as found in the commercial varieties. Thus, screening for CBN resistance

and tolerance among the acceptable breeding lines and plant introductions of the cultivated species are important to widen the source for CBN resistance and tolerance germplasm.

Intraspecies and Interspecies Cross Compatibility

Generally, species in the section *Calomystrium* are closely related taxonomically (Kamemoto and Kuehnle, 1996). Using the male sterile cultivar, 'Uniwai', intrasectional hybrids were obtained only with closely related species of the section *Calomystrium*: *A. formosum, A. kamemotoanum, A. lindenianum, A. nymphaeifolium, A. roseospadix*, and *A. hoffmanii*. Intersectional species hybrids of *Anthurium* in section *Calomystrium* with other sections reported were section *Belolonchium, Cardiolonchium, Semaeophyllium*, and three species in section *Porphyrochitonium* (*A. amnicola, A. antioquiense*, and *A. antrophyoides*).

1.7. Relationship between Plant Nutrition and Nematode Tolerance and Resistance

Changes in Plant Nutrition in Response to Nematode Infection

Plants infected by nematodes often exhibit symptoms of nutrient deficiency. Evidence of changes in host plant nutrition in response to nematode infection have been reported. For example, potato plants infected with potato cyst nematodes contained less P and K, and more Ca than the uninfected plants (Trudgill et al., 1975). In soybean cyst nematode infected soybean, root concentration of K and Mg decreased and Ca and P increased whereas leaf concentration of Mg and Ca increased (Blevins et al., 1995).

Relation of Nematode Tolerance to Plant Nutrition

With the evidence of changes in plant nutrition affected by the infection by nematodes, a relation between nematode tolerance and plant nutrition would be expected. Yield (indication of tolerance) of infected plants was positively correlated with concentration of K while negatively correlated with concentration of Ca of the uninfected

plants (Evans and Franco, 1979). K is absorbed into roots through the symplasm; but Ca is transported through the apoplasm (Clarkson, 1988). Therefore Ca uptake is restricted to the younger parts of the roots and its rate of uptake is affected by transpiration (Clarkson, 1988). Plants that use water inefficiently accumulate a greater Ca concentration in the roots (Trudgill et al., 1975). However, there was evidence that early maturing cultivars of potato accumulated more Ca in the dry matter than later maturing cultivars (Trudgill and Cotes, 1983). Therefore correlation between nematode tolerance and plant tissue Ca concentration should be assessed using plants of the same maturity rate (Evans and Haydock, 1990).

In other cases, nematode tolerance is related to cation ratio. For example, a decreased K/Ca ratio occurs in a potato cultivar with low tolerance to the potato cyst nematode (Evans and Franco, 1979). According to Reinbott and Blevins (1991), Ca and Mg uptake and translocation mechanisms are similar under the same level of P treatments, but Mg and Ca uptake are antagonistic to K uptake. High K uptake depressed the translocation of Mg and Ca from roots to shoots. With increasing P, K decreased, and Mg and Ca concentrations increased, which resulted in decreased in K/(Ca+Mg). This principle was also applicable to the K/Ca relation with nematode tolerance.

Blenkinshop showed that heavy dressings of K fertilizer reduced yield loss due to cyst nematode in potatoes grown on soils with low K reserves (in Evans and Haydock, 1990). This is because K regulates stomatal aperture; supplying K fertilizer to a plant might decrease plant transpiration rates and improve efficiency of water use (Brandbury and Malcolm, 1977).

Relation of Nematode Resistance to Plant Nutrition

K applications were found to decrease citrus nematode, *Tylenchulus semipenetrans*, populations in roots and soil (Rabeh and Sweelam, 1990). A high (Ca+Mg)/(Na+K) ratio was positively correlated with alfalfa resistance to *Ditylenchus dipsaci* (Sherwood and

Huisingh, 1970). Furthermore a high (Ca+Mg)/K was negatively correlated with resistance of various crops to the root-knot nematode, *Meloidogyne incognita* (Bains et al., 1984).

Vesicular-Arbuscular Mycorrhizae (VAM) Effects

Vesicular-arbuscular mycorrhizae (VAM) are symbiotic organisms associated with roots that increase the plant's ability to absorb phosphorus (P), minor elements and water (Smith, 1987). This symbiosis has shown the potential to limit yield losses due to nematodes by either improving nematode tolerance or resistance (Cooper and Grandison, 1986).

In many studies, VAM was found to increase plant nematode tolerance. This includes studies of rough lemon response to *R. similis* (Smith and Kaplan, 1988), and alfalfa, cotton and soybean to *M. incognita* (Cooper and Grandison, 1986; Francl, 1993; Hussey and Roncadori, 1982). In other studies, VAM induced resistance in cotton to *M. incognita* and *P. branchyurus* (Hussey and Roneadori, 1982), and in tomato and white clover to *M. hapla* (Cooper and Grandison, 1986).

Further studies in this field continue to search for the mechanism of VAM's ability to increase plant tolerance or resistance to nematodes. Again, variation occurs in different hosts. The two most controversial mechanisms proposed are the increased P absorption due to VAM and the possible antagonism between nematodes and mycorrhizal fungi.

In support for the hypothesis that the VAM-induced increase in P uptake is responsible for increased plant tolerance or resistance to nematodes, Hussey and Roncadori (1983) found that increasing the P available to nematode-infected plants offset the nematode damage symptoms. A similar result was reported by Smith and Kaplan (1988) with rough lemon citrus infected by the burrowing nematode. High P was usually found to increase nematode reproduction. However, in a study on tomato and white clover infected by rootknot nematode, *M. hapla*, nematodes still depressed shoot growth even though the

shoot P content was uniformly high (Francl,1993). The author speculated that high P also resulted in Zn deficiency (Francl,1993).

Evidence for the hypothesis that VAM antagonism with nematodes is responsible for host plant tolerance or resistance is as follows. Cooper and Grandison (1986) found that nematodes were not present in root segments where the level of internal mycorrhizal infection was greater than 10%. This suggests that VAM competes with the nematode for space and feeding sites. A VAM, *Glomus fasciculum,* was found to parasitize *Heterodera glycines* eggs, but the level of parasitism was considered insufficient to negatively affect nematode activity (Smith, 1987). Harley and Smith (1983) estimated that a mycorrhizal symbiosis utilized up to 15% of host photosynthates and competed for host nutrition with nematodes.

The other hypotheses on VAM function in increasing plant tolerance or resistance to nematodes are summarized by Smith (1987) as follows:

> (1) Improved nutrition absorption other than P, including Ca, Cu, Mn, S and Zn.
> (2) Increased water uptake.
> (3) Alteration in root exudation. McArthur and Knowles (McArthur and Knowles, 1992) showed that VAM leachate contained higher concentrations of phenolics, which might contribute to plant resistance to nematode.
> (4) Physical alteration of the plant. According to McArthur and Knowles (1992), VAM can induce papilla-like thickening in epidermal cells and increase the activity of cell-wall bound peroxidase that is involved in lignin formation, and thus reduce nematode infection.
> (5) Physiological alteration. McArthur and Knowles (1992) showed that VAM decreased ACC oxidase activities, and they hypothesized that these physical and physiological changes could increase ethylene synthesis and thus resistance to pathogens.

1.8. Objectives of Research

The overall objective of this thesis is to examine genetic and cultural control of *R. citrophilus* in *Anthurium.* The five topics examined are listed as follows:

Cultural Control through Manipulation of the Growing Medium

Quarantine restriction in Japan, California, Texas and Arizona prohibit entry of potted plants contaminated by burrowing nematode. Only limited soil-free potting media are approved for export. Control of *R. citrophilus* in *Anthurium* through manipulation of the growing medium should thus focus on soil-free potting media. Due to the difficulties in controlling the source of nematode contamination, soil-free media are not guaranteed to be nematode-free. Identification of soil-free media unfavorable for burrowing nematode infection on *Anthurium* is of interest.

Distribution of Burrowing Nematode in *Anthurium*

Burrowing nematodes are found predominantly in root tissue, and their presence in shoot tissue has not been reported. However, *Anthurium* shoot top cuttings from 30.5 cm above the medium level, grown in nematode free medium, with aerial roots tested to be free of burrowing nematodes at the time of planting, were later infected by burrowing nematodes (Goo, 1995). As the shoot (top) cutting method is common for *Anthurium* propagation, and *R. citrophilus* is known to enter the stele of citrus roots (DuCharme, 1959), it was suspected in this case that the nematode might have invaded the stele from the root and penetrated into the stem tissue. Therefore, one research objective is to sample roots, stems, and petioles for the presence of the burrowing nematode in inoculated *Anthurium*.

Screening *Anthurium* Cultivars for Burrowing Nematode Resistance and Tolerance

Nematode resistance is independent of tolerance (Trudgill, 1985). Both resistance and tolerance have their advantages and disadvantages in plant protection. Integration of resistance and tolerance to control burrowing nematode would offer a new tactic in burrowing nematode control. A rapid and reliable method to screen *Anthurium* for burrowing nematode resistance and tolerance is essential. Identification of differential screening parameters and of standard tolerant, intolerant, susceptible and resistant

references is critical for development of an effective screening system for future breeding. A reliable screening system should also be able to differentiate the relatively tolerant from the relatively resistant cultivars.

Relationship between Burrowing Nematode Resistance and Tolerance and Plant Nutrition in *Anthurium*

Variations in nutrient content or ratios of cations have been reported in relation to plant resistance and tolerance to nematodes (Evans and Franco, 1979; Trudgill, 1975; Blevins et al., 1955; Clarkson, 1988; Sherwood and Huisingh, 1970; Bains et al., 1984). Among the nutrient elements or cation ratios related are P, K, Mg, Ca, K/Ca, (Ca+Mg)/K or (Ca+Mg)/(Na+K). Understanding the relationship between burrowing nematode host resistance and tolerance with these nutrient elements or cation ratios will 1) aid in future burrowing nematode resistance and tolerance selection in *Anthurium* as a selection marker; 2) provide information on increased burrowing nematode resistance and tolerance through fertilization. Therefore, possible correlations between *Anthurium* tolerance and resistance to burrowing nematode and 11 plant essential elements will be examined.

Screening for Burrowing Nematode Resistance and Tolerance in *Anthurium* Species

The important commercial *Anthurium* cultivars are *A. andraeanum* Linden ex André for cut flower production in Hawaii, and *A. scherzerianum* Schott for potted plants in Europe. No burrowing nematode resistant *Anthurium* cultivars have been reported. Screening for burrowing nematode resistance and tolerance from related *Anthurium* species might broaden the genetic base for burrowing nematode resistance and tolerance improvement.

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Chapter 2. Evaluating Soilless Media Suitable for *Anthurium* Growth and Unfavorable for Burrowing Nematode Damage

2.1. Abstract

Four soilless media; 1:1 (v/v) pine bark compost-perlite, cinder, 2:1 (v/v) cinder-peat, and a 1:2:1 (v/v/v) rockwool-cinder-peat, were evaluated in the greenhouse for their effects on the growth of *Anthurium* cultivars 'Alii' and 'Midori' and on the population increase of citrus burrowing nematode (CBN). Nine months after inoculation with 2000 CBN per plant, root and shoot weights of 'Alii' differed among media. Cinder medium reduced CBN damage in terms of root fresh and dry weight, root vigor, shoot damage, number of new leaves and flower number in 'Alii'. The effects were more pronounced in 'Alii' than in 'Midori'. Since uninoculated plants grew better in pine bark compost-perlite and rockwool-cinder-peat, the effect of the medium was not due to improved plant growth. Nematode number was lowest in shoot tissue of 'Midori' grown in cinder, suggesting that cinder reduced nematode infection. Orthogonal contrast analysis showed that the effect of the medium on nematode damage might be due to organic matter content.

2.2. Introduction

Citrus burrowing nematode (CBN), *Radopholus citrophilus* Huettel, Dickson, Kaplan, is a common pest of *Anthurium* (Hara et al., 1988) and causes anthurium decline. Reduction in flower yield can reach 50% among infected plants (Aragaki and Apt, 1984). Fenamiphos is an effective nematicide to control CBN in *Anthurium* (Hara et al., 1988), however its application rate has recently been reduced from 15G to 10G. Additional non-pesticide methods of control are highly desirable.

Manipulation of the growth medium might offer a tactic for burrowing nematode management, as many studies have shown a close interaction between nematodes and soil characteristic. CBN migrated longer distances and reproduced best in well-drained soils

(O'Bannon and Ford, 1976). Infection of plants by the citrus nematode, *Tylenchulus semipenetrans* was enhanced in those media with higher organic matter and moisture contents (Van Gundy et al., 1964). Moreover, since nematode eradication in *Anthurium* production sites is difficult (Goo, 1995), media with characteristics unfavorable for nematode damage may facilitate crop production.

Quarantine restrictions in Hawaii's primary plant export destinations, namely Japan, California, Arizona, and Texas, prohibit entry of all potted plants contaminated by the burrowing nematode (L. Wong, Hawaii State Department of Agriculture, personal communication; Evans and Greczy, 1995). Only limited soilless media, such as peat, sphagnum, bark charcoal, perlite, vermiculite, rockwool, pumice, and volcanic cinder are permitted for exported potted plants. Many of these appear suitable for *Anthurium*, which grows well in media that is well-aerated and has a high water holding capacity (Higaki et al., 1994). More information is needed on soilless media that reduce anthurium decline while meeting quarantine restriction requirements. The growing medium should also enhance plant growth, as the vigor of the plant may increase its tolerance towards nematode infection.

The purpose of this study was to identify soilless media, and its characteristics, effective in controlling anthurium decline; and to evaluate whether the effect is due to an increase in plant vigor or to a decrease in nematode infection.

2.3. Materials And Methods

Plant Materials and Growing Media

Twelve uniformly sized *Anthurium andraeanum* 'Midori' and 'Alii' plants were transplanted into 3.8 liter black plastic pots filled with 3 liters of one of four media, 1:1 (v/v) #3 perlite-pine bark compost (Cascade Forest Products Inc., Arcata, CA); volcanic cinder (0.64 to 1.25 cm size); 2:1 (v/v) cinder-peat moss; or 1:2:1 (v/v/v) cinder-peat moss-

rockwool (medium size, 1 part water resistant and 1 part water absorbent; Capogro, Chicago, IL). Plants were grown on a 60-cm raised bench under 65% shading in a glasshouse at the University of Hawaii at Manoa. Plants were hand-watered and alternately fertilized in each pot with 8 g of slow release Osmocote (14-14-14) or organic fertilizer (5-15.5-14.5; Bandini Pro., Los Angeles, CA) every 3 months. Plants were grown in these conditions for 1 month prior to nematode inoculation. 'Alii' and 'Midori' plants were about 30 cm and 20 cm tall, respectively, and with established root systems at inoculation.

Nematode Inoculation

Nematode inoculum was obtained from callused alfalfa root tissue maintained in vitro (Ko et al., 1996). Tissue was placed in a mist chamber for 24 hours to collect the nematodes. The number of nematodes was determined and the inoculum was adjusted to give approximately 2000 CBN in 10 ml of water. The inoculum was applied in a 1.5-cm radius around the base of each plant.

Pots were arranged in random complete blocks with inoculation time serving as a block. Plants were separated into three blocks sequentially and inoculated at weekly intervals. Each block of plants consisted of four media and two nematode treatments, i.e., inoculated and uninoculated (control), with two replicates for each treatment in a block.

Evaluation Methods

The experiment was conducted for 9 months with weekly assessment of the number of new leaves, chlorotic leaves, yellow leaves, necrotic leaves, and flowers. Plant vigor was evaluated by new leaf numbers, flower numbers, ratio (inoculated / uninoculated) of plant fresh and dry weights, root vigor index, and shoot damage index. A root vigor index was derived by:

Root Vigor Index = root fresh weight × root vigor level

where root vigor level was defined as follows: Level 1–black and rotten; Level 2–brown and corky; Level 3–fresh but slender; and Level 4–fresh and fleshy. A shoot damage index was defined as:

$$\text{Shoot Damage Index} = (\%\ \text{level 1 leaves} \times 1) + (\%\ \text{level 2 leaves} \times 2) + (\%\ \text{level 3 leaves} \times 3) + (\%\ \text{level 4 leaves} \times 4)$$

where leaf damage levels were defined as follows: Level 1–chlorotic, Level 2–yellow, Level 3–partly necrotic, and Level 4–totally necrotic. At the end of the experiment, shoot and root fresh and dry weights were determined. In addition, nematodes were extracted from the media and from plant tissues in a mist chamber. The logarithm value of nematode number was used to evaluate nematode reproduction.

Analysis of variance of the three-way factorial experimental design (media × cultivar × nematode) was conducted to determine effects of media and cultivars on plant growth and nematode reproduction. The Waller-Duncan multiple range test was used to analyze differences between treatments. Orthogonal contrasts were used to analyze the effects of organic matter, cinder, and rockwool on CBN damage to *Anthurium.*

2.4. Results

Media Suitable for Reducing Anthurium Decline

Among the four media tested, 'Alii' grown in cinder showed the least damage from CBN infection in terms of the root fresh and dry weight ratios (Fig. 2.1A,B), shoot fresh and dry weight (P>0.05; Fig. 2.1C,D) shoot damage index ratio (Fig. 2.1E), root vigor index ratio (Fig. 2.1F), number of new leaves (Fig. 2.1G), and flower number (Fig. 2.1H). Although 'Midori' had a stronger root system than 'Alii' as indicated by its higher root fresh and dry weights, and root vigor index (Table 2.1), inoculated 'Alii' grown in cinder was able to reduce nematode damage to such an extent that root fresh and dry weights and root vigor index ratios became higher than those of 'Midori' (Fig. 2.1C,D,

and F). In 'Midori', media had no effect on nematode infection in terms of plant growth (P>0.05). Only new leaf formation was affected, with the number of new leaves higher in pine bark compost-perlite and in cinder than in the other media (P=0.04) (Fig. 2.1G). Shoot and root damage symptom of the inoculated 'Alii' and 'Midori' in different media were shown in Fig. 2.2.

Comparison of the uninoculated root systems of both cultivars showed that 'Alii' had fleshier roots with fewer lateral roots while 'Midori' had finer roots with many lateral roots (Fig. 2.3). Additional observations showed that infected 'Alii' tended to results in senesces leaves whereas 'Midori' tended to express chlorotic leaves.

Effect of Medium on Anthurium Decline

Uninoculated 'Alii' and 'Midori' grew better in media with higher organic matter content, for example, pine bark compost-perlite and rockwool-cinder-peat (Table 2.1). The effects were more pronounced in the roots than in the shoots, as the Waller-Duncan k-ratio t-test (k=100) showed that only root fresh and dry weight and root vigor index differed among media (P<0.05; Table 2.1). Root vigor of the uninoculated 'Alii' and 'Midori' growing in different media were shown in Fig. 2.3.

Nematode reproduction was affected either by the root fresh weight or the organic matter and moisture content of the medium. Total nematode number from root, shoot, and medium combined was highest in 'Alii' cultured in cinder (P=0.01; Fig. 2.4). This medium provided the highest root fresh weight (Fig. 2.1.C). In the case of 'Midori', where there was no difference in root vigor among media, nematode numbers were higher in rockwool-cinder-peat, cinder-peat, and pine bark compost-perlite but not significantly different from cinder (P>0.05; Fig. 2.4). Nematode numbers in shoots of 'Midori' cultured in cinder were lower than those cultured in rockwool-cinder-peat (P=0.074).

Characteristics of the Media Effective in Controlling Anthurium Decline

The medium effect in 'Alii' was due to either organic matter or cinder composition. Root fresh weights and the root vigor index showed significant differences ($P<0.05$) for the organic matter or cinder orthogonal contrast (Table 2.2). Although root dry weight showed differences ($P<0.05$) for the compost or rockwool contrasts, a higher difference ($P<0.01$) was seen for the organic matter or cinder contrast. In the case of 'Midori', with no growth differences among media, root fresh weight and vigor were different among media with or without rockwool (Table 2.1). This result is consistent with the higher number of nematodes in the rockwool-cinder-peat medium for 'Midori' (Fig. 2.4).

2.5. Discussion

Effect of Medium on Anthurium Decline

Cinder medium reduced CBN damage on *Anthurium* 'Alii'. This effect was not likely due to plant growth enhancement by cinder since both uninoculated cultivars grew poorly in cinder compared to other media with higher organic matter content. Rather, a reduction in CBN damage was likely due to a decrease in nematode reproduction. Nematode reproduction depends on many factors, including root vigor and the organic and moisture contents of the medium. It is possible that during the early CBN infection period, where 'Alii' root vigor did not differ among media, nematode reproduction resembled that in 'Midori', with significantly fewer nematodes invading shoots of plants grown in cinder. Thus, in turn, resulted in the lowest CBN damage among media.

The indifferential effects of medium on the two cultivars tested might be related to differences in root vigor accompanied by differential tolerance limits for CBN infection. Detailed studies on the response of potato cultivars to potato cyst nematode (Evans and Franco, 1979; Trudgill, 1980; Trudgill and Cotes, 1983; Trudgill et al., 1975a; Trudgill et al., 1975b; Van Gundy et al., 1964) gave clear indications that root vigor was an important

factor responsible for nematode tolerance. Trudgill (1986) also demonstrated that the degree of nematode tolerance was determined by the grafting stock in potato. Even though nematode infection was higher in media with high organic matter content, it is plausible that 'Midori' was able to tolerate nematode damage because 'Midori' is a CBN tolerant cultivar (Wang et al., 1996). This would account for the lack of pronounced medium effect on nematode reproduction observed 'Midori'.

Characteristics of the Media Effective in Controlling Anthurium Decline

Orthogonal contrast analysis of the medium components suggested that reduction of nematode damage in cinder is due to lack of organic matter. This result is consistent with previous studies which suggested that organic matter created a thin layer over roots that favored citrus nematode infection (O'Bannon, 1967, Van Gundy et al., 1964). Compost–perlite, cinder–peat, and rockwool–cinder–peat contain organic matter that could create such a thin layer on *Anthurium* roots, thus enhancing nematode infection. Moreover, cinder might also reduce nematode infection by ensuring good water drainage. Nematodes that have not infected plant roots or exit the roots might have been washed away with the irrigation water more readily in cinder than in other media.

In summary, soilless media suitable for *Anthurium* growth are pine bark compost-perlite and rockwool-cinder-peat. However, soilless medium unfavorable for nematode damage is cinder. Therefore, cinder is recommended for *Anthurium* culture under conditions of nematode presence. The ability of cinder medium to significantly improve root vigor of a cultivar inoculated with CBN suggests that selection of an appropriate growing medium can provide a supplemental method to manage anthurium decline problems of intolerant cultivars. Such cultivars are currently being identified in our breeding program.

Table 2.1. Root and shoot fresh and dry weights, and root vigor index (root vigor level × root fresh weight) of *Anthurium* 'Alii' and 'Midori' free of *Radopholus citrophilus* in four media, 1:1 (v/v) pine bark compost–perlite; cinder; 2:1 (v/v) cinder–peat; and 1:2:1 (v/v/v) rockwool–cinder–peat .

	Fresh weight (g)		Dry weight (g)		Root vigor
Medium	Root	Shoot	Root	Shoot	index
'Alii'					
compost–perlite	49.03 a	111.28 ns	6.27 ab	16.71 ns	6.27 ab
cinder	16.82 c	85.00 ns	2.76 b	14.41 ns	2.76 b
cinder–peat	26.73 bc	74.07 ns	4.61 ab	10.45 ns	4.61 ab
rockwool–cinder–peat	40.28 ab	106.81 ns	7.95 a	16.51 ns	7.95 a
'Midori'					
compost–perlite	53.33 xy	105.30 ns	6.42 ns	20.07 ns	228.80 ns
cinder	25.47 y	90.07 ns	5.32 ns	14.87 ns	91.63 ns
cinder–peat	41.96 xy	143.13 ns	7.10 ns	26.58 ns	172.71 ns
rockwool–cinder–peat	60.32 x	150.23 ns	10.10 ns	55.10 ns	221.72 ns

[z]Data are means of six replications. Means followed by the same letter in the same column are not different within cultivar ($P<0.05$), according to the Waller-Duncan k-ratio t-test (k=100).

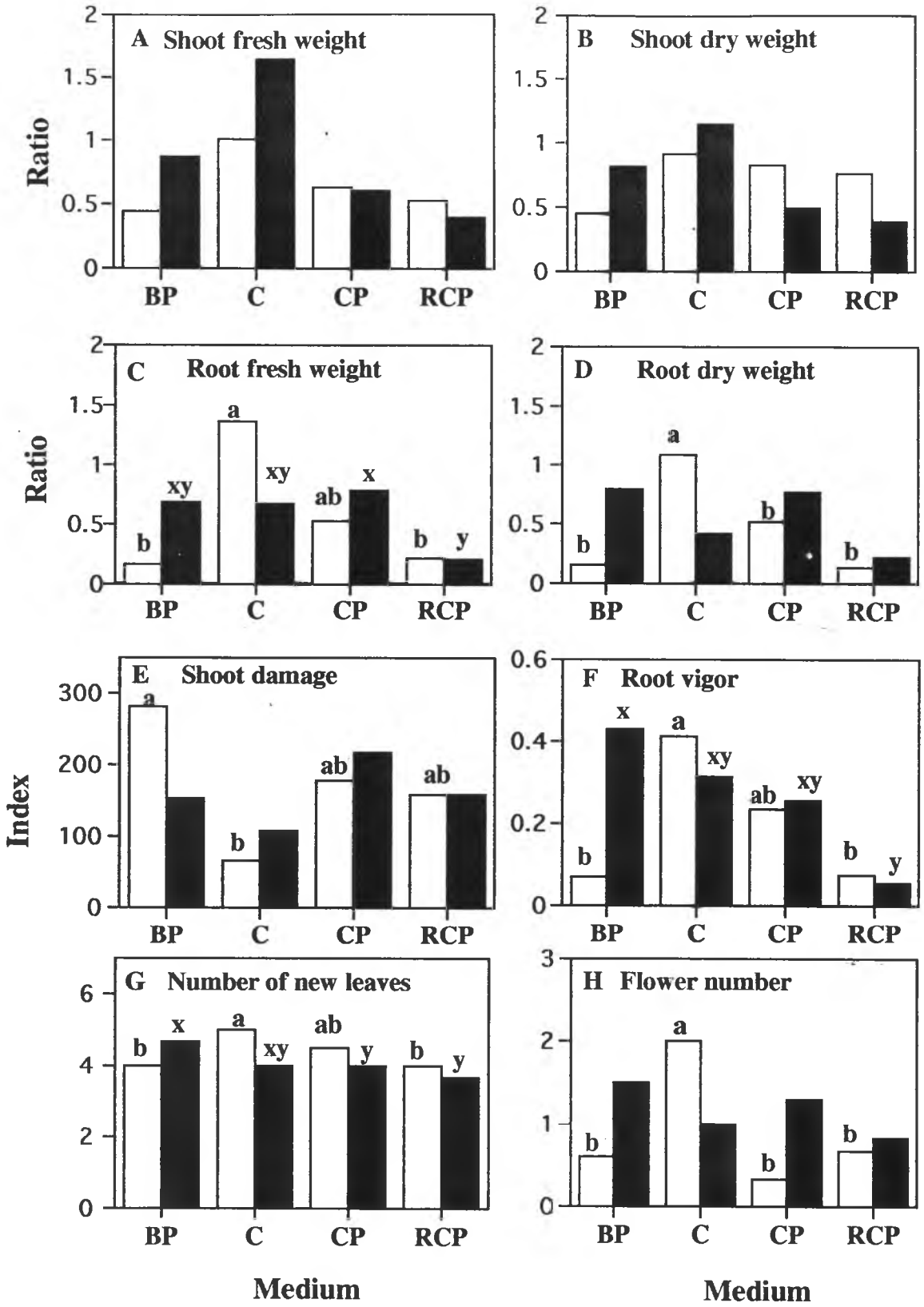
ns=not significantly different.

Table 2.2. Orthogonal contrast of media components in root fresh and dry weights, and root vigor index[z] for 'Alii' and 'Midori' inoculated with *Radopholus citrophilus*.

Cultivar	Contrasts	Root fresh weight	Root dry weight	Root vigor index
'Alii'	compost *vs.* others	ns	*	ns
	cinder *vs.* others	*	**	**
	peat *vs.* others	ns	ns	ns
	rockwool *vs.* others	ns	*	ns
	organic matter *vs.* others	**	**	**
'Midori'	compost *vs.* others	ns	ns	ns
	cinder *vs.* others	ns	ns	ns
	peat *vs.* others	ns	ns	*
	rockwool *vs.* others	*	ns	*
	organic matter *vs.* others	ns	ns	ns

[z]root vigor index = root vigor level × fresh root weight.
ns, *, ** Nonsignificant or significant at $P< 0.05$ or $P<0.01$ respectively.

Fig. 2.1. (A) Shoot fresh weight ratio, (B) Shoot dry weight ratio, (C) Root fresh weight ratio, (D) Root dry weight ratio (E) Shoot damage index, (F) Root vigor index, (G) Number of new leaves, and (H) Flower number of *Anthurium* 'Alii' (white bar) and 'Midori' (black bar) in four media, 1:1 (v/v) pine bark compost-perlite; cinder; 2:1 (v/v) cinder-peat; and 1:2:1 (v/v/v) rockwool-cinder-peat, abbreviated as BP, C, CP, and RCP, respectively, 9 months after inoculation with *Radopholus citrophilus*. Ratio refer to inoculated/uninoculated values; shoot damage index = Σ % of each of the four leaf damage levels × level of leaf damage; root vigor index = root vigor level × root fresh weight. Bars with the same letter are not different according to the Waller-Duncan k-ratio t test (k=100), where a, b and c, are used for 'Alii' and x, and y are used for 'Midori'. Data are means of 6 replicates.


A Shoot fresh weight
B Shoot dry weight
C Root fresh weight
D Root dry weight
E Shoot damage
F Root vigor
G Number of new leaves
H Flower number
Ratio
Index
BP
C
CP
RCP
Medium

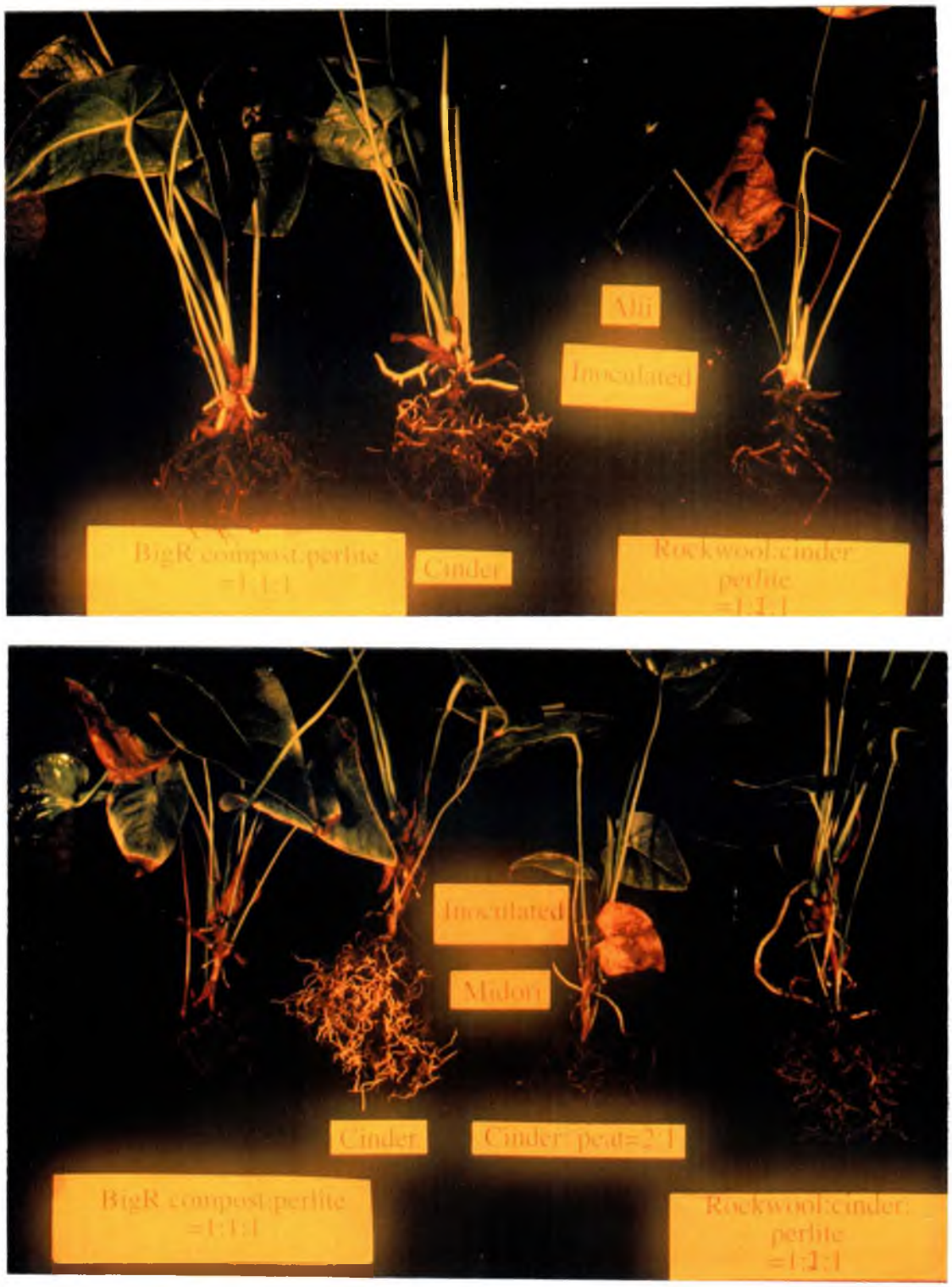


Fig. 2.2 Shoot and root damage symptom of the *Radopholus citrophilus* inoculated 'Alii' and 'Midori' grown in different media.

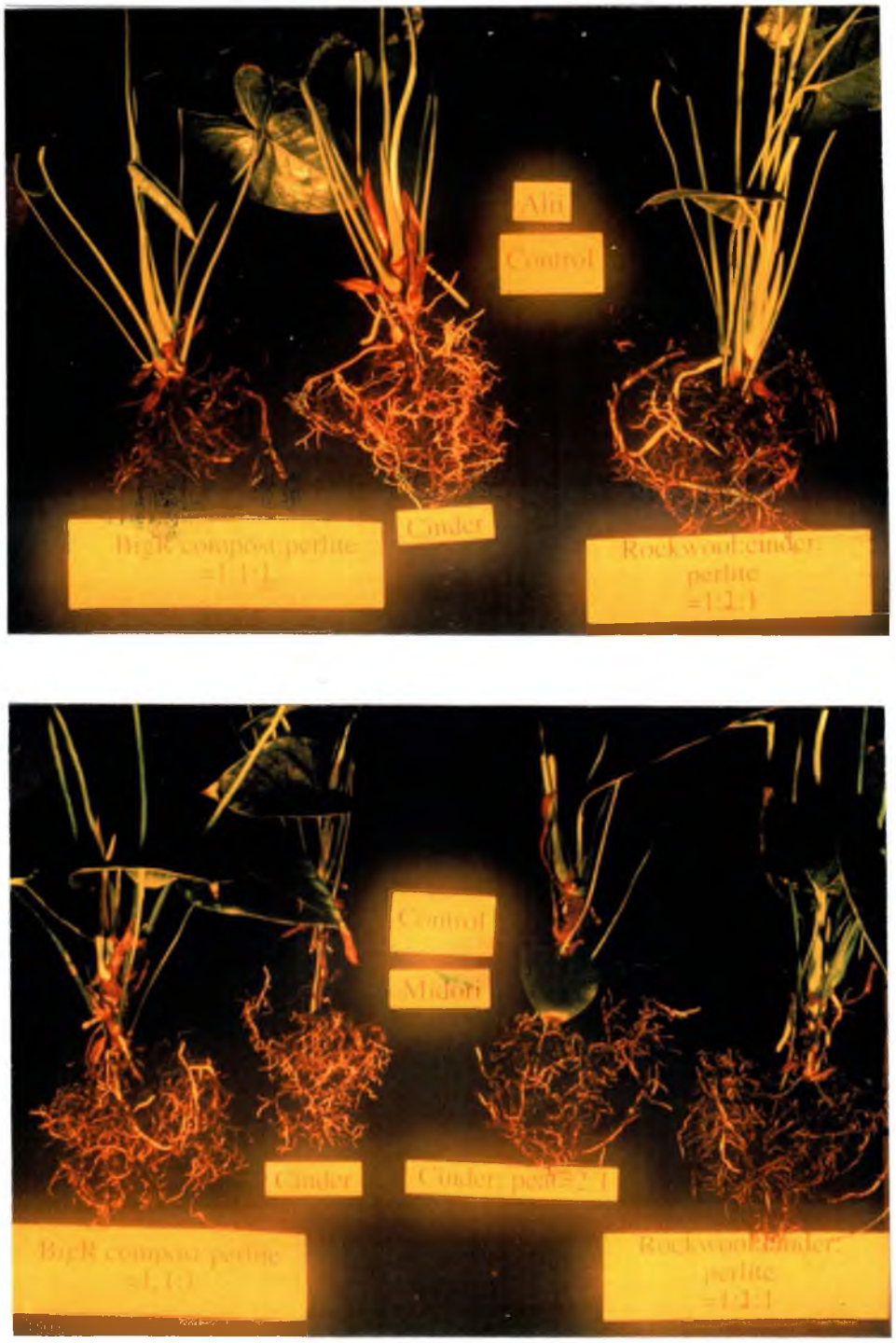


Fig. 2.3. Root system of uninoculated 'Alii' and 'Midori' grown in different media.

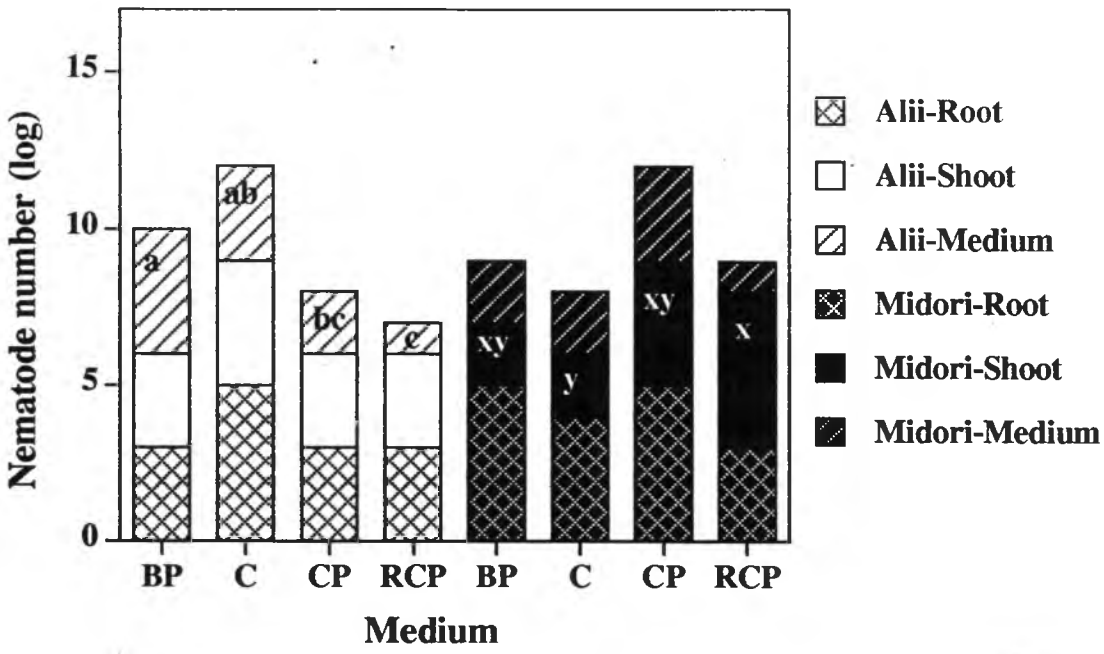


Fig. 2.4. Nematode number (log value) extracted from root, shoot, and medium, of *Anthurium* 'Alii' (white bar) and 'Midori' (black bar) in four media 1:1 (v/v) pine bark compost-perlite, cinder, 2:1 (v/v) cinder-peat, and 1:2:1 (v/v/v) rockwool-cinder-peat, abbreviated as BP, C, CP, and RCP, respectively, 9 months after inoculation with *Radopholus citrophilus*. Section in bars with the same letter are not different according to the Waller-Duncan k-ratio t test (k=100), where a, b and c, are used for 'Alii' and x, and y are used for 'Midori'. Data are means of 6 replicates.

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Chapter 3. Screening for Burrowing Nematode, *Radopholus Citrophilus*, Tolerance and Resistance in Commercial *Anthurium* Hybrids

3.1. Abstract

A rapid method to screen *Anthurium* for burrowing nematode resistance and tolerance in vitro was developed using 17 genetically distinct *Anthurium* cultivars. Based on nonparametric data analysis, tolerance and resistance were found to be independent traits to be evaluated separately. An effective parameter for tolerance evaluation was ranking of relative leaf retention, whereas an effective parameter for resistance evaluation was the nematode reproduction, log(Rf+1). Rf, nematode reproductive factor, is the final nematode population divided by initial nematode population. A comparison of the ranking sequences of leaf retention with nematode reproduction in vitro clustered the cultivar responses to burrowing nematode infection into four groups: intolerant and resistant, moderately tolerant but susceptible, intolerant and susceptible, tolerant and susceptible. 'Ozaki' was identified as intolerant reference, 'Nitta' as susceptible reference. 'Blushing Bride' was the most tolerant cultivars among the cultivars screened but it was not an ideal cultivar for tolerant reference due to its low vigor. Leaf retention of inoculated plants identified 'Midori' as the most vigorous cultivar. No resistant cultivar could be identified under in vitro screening condition due to nematode population crashes in the highly damaged plants. Results for ex vitro tolerance screening were correlated with that for in vitro tolerance screening but failed to screen for nematode resistance. Future screening for burrowing nematode resistance in *Anthurium* should be based on a nematode parasitism index by using 'Nitta' as a reference standard; tolerance should be based on relative leaf retention or relative weight gain of 'Midori'.

3.2 Introduction

Burrowing nematode, *Radopholus citrophilus*, is one of the three common pests reducing *Anthurium* production (Hara et al., 1988). *Anthurium* flower yield can be reduced up to 50% (Aragaki and Apt, 1984). Fenamiphos is an effective nematicide to control *R. citrophilus* in *Anthurium* (Hara et al., 1988), however its application rate has recently been reduced from 15 G to 10 G. Additional methods of control are highly desirable.

Breeding for resistance is becoming a major component for pest management (Roberts, 1992). Resistance offers more effective control of *Globedera rostochiensis* and *G. pallida* on potato than chemical treatment (Trudgill et al., 1978; Gurr, 1992). While current management of nematodes is largely based on chemical controls, many nematicides may be banned in the near future. Other methods of management, including biological control, physical control, crop rotation, soil amendment or field sanitation are unable to eradicate nematodes. Plant host resistance and tolerance thus complements other integrated pest management strategies and provide a more direct control of the disease.

Host plant resistance enables the plant to hinder pathogen invasion, development or multiplication. Tolerance enables the plant to withstand nematode infection without suffering undue damage (Trudgill, 1985). In nematology, resistance may be independent of tolerance, therefore, plants can be categorized into four groups: tolerant and resistant, tolerant and susceptible, intolerant and susceptible, and intolerant and resistant (Trudgill, 1985).

Nematode resistance can place selection pressure on nematode biotypes. According to the gene-for-gene theory, nematode virulence genes (genes conferring the ability to reproduce on a plant with one or more major resistance genes; Trudgill, 1985) may predominate and lead to selection of resistance breaking nematodes biotypes (Parrott, 1981; Turner and Perry, 1983; Roberts, 1992; Riggs and Winstead, 1959). Evidence of this resistance-breaking is not only confined to single-gene resistance systems but also occurs

in polygenic resistance systems (Turner et al., 1983; Roberts, 1992). Nematode tolerance however is race independent (Reese et al., 1988) and thus could provide a longer term strategy to reduce nematode pest problems. However, the disadvantage of tolerance as a pest management stratergy is that it might allow the nematode population to build up to an intolerable level.

No screening for burrowing nematode resistance and tolerance in *Anthurium* has been conducted. Most of the current screening for nematode resistance has been in the field. Anecdotal burrowing nematode resistance has been observed in *Anthurium* (Table 1). Nonuniformity of nematode infestation in the field, seasonal fluctuations of nematode reproduction, and complications due to polyspecies nematode communities have caused an unreliability in field screenings (Boerma and Hussey, 1992). In vitro screening, however, can lessen these problems by controlling the inoculum level, shortening the screening period, and by avoiding seasonal effects (Boerma and Hussey, 1992). In vitro screening for *Meloidogyne incognita* resistance in peach shortened the time needed for evaluations from 3 years in the field to 5 weeks in vitro (Huettel and Hammerschlag, 1993). Results from in vitro screening of self-rooted peach cultivars and rootstocks resistance to *M. incognita* with hormone-free medium were similar to those obtained under field conditions (Huettel and Hammerschlag, 1993).

The objectives of this experiment were to develop a reliable method to screen for *R. citrophilus* resistance and tolerance in *Anthurium* and to identify standard susceptible and intolerant *Anthurium* cultivars for future screening reference. Comparison between in vitro and ex vitro screening was conducted to evaluate if the plant response would be consistent in either growth condition.

3.3. Materials and Methods

In vitro screening

Seventeen Hawaiian *Anthurium* cultivars (Table 3.1.) were maintained in tissue culture medium H1 (Kunisaki, 1980) lacking BA and solidified with 0.7% Difco Bacto agar. 'Ozaki' was cultured on the same medium but with macronutrient concentration reduced by one-eight. Four 2-cm-tall plantlets per cultivar were transferred into a GA-7 box (Sigma, St. Louis, Mo) containing the same medium, but solidified with 0.3% Gelrite and inoculated with nematodes 2 weeks later. Plants were cultured at 25±2°C under illumination of 14.34 $\mu E.m^{-2}.sec^{-1}$ photoflux density provided by cool white and Gro-lux Sylvania flurescent lamps (GTE Corp., Danvers, MA).

Radopholus citrophilus, population HA11, collected from Paradise Pacific Farm in Kurtistown, Hawaii and subsequently maintained on alfalfa root callus (Ko et al., 1996) was used as the inoculum source. Numbers of nematode per unit length of alfalfa callus were estimated by counting the nematodes extracted in a mist chamber. Callus pieces with approximately 400 nematodes were placed in each GA-7 box. The same length of root callus without nematodes was inserted into the uninoculated boxes to serve as controls.

A random complete block design was used with five replicates repeated over 10 weeks. Plants were harvested 3 months after inoculation, rated for disease symptoms, and placed in a mist chamber for 7 days to extract nematodes. Plant damage was expressed in a symptom index (SI) which was derived from the summation of percentages of leaf damage levels multiplied by the level of leaf damage. Leaf damage levels were assigned as: Level 1=brown spots, 2=chlorotic, 3=partly yellow, 4=completely yellow, 5=partly brown, 6=completely brown.

Analysis of variance was used to analyze the parametric variables (relative SI, relative leaf retention, and nematode reproduction) and their corresponding nonparametric variables (ranking of relative SI, ranking of relative leaf retention and ranking of nematode

reproduction). Relative SI is the difference between inoculated and uninoculated treatment; relative leaf retention is the percentage green leaves retained on inoculated plants with respect to the uninoculated plants; and nematode reproduction rate is log(Rf+1), where Rf is final nematode population divided by initial nematode population. Analysis of nonparametric variables was suggested by Eskridge (1995) and Phillips (1984). Waller-Duncan's multiple-range test was used to compare differences among cultivars and treatment means. Means of the ranking of relative SI for each cultivar were plotted against means of ranking of nematode reproduction. The Fastclus procedure of the SAS program (SAS Institute, 1985) was used to cluster data points in the plot into four groups. Analysis of variance of the leaf retention of inoculated and uninoculated plants was also conducted. Correlation analysis among nematode reproduction, relative symptom index and relative leaf retention was done.

Ex vitro screening

A trial to corroborate in vitro results was conducted in a 65% shaded greenhouse. Tissue cultured plantlets of the 17 cultivars tested in vitro were transplanted into a Big-R pine bark compost (Cascade Forest Product, Arcata, CA) and perlite #3 (1:1) potting mix in 7.5 cm-diameter clay pots after removal from tissue culture flasks. The experimental design was the same as in vitro screening except that only four replicates were used. Plants were fertilized weekly with 50 ml per pot of a 500 µg/l 23-19-17 liquid fertilizer.

A *R. citrophilus* preparation extracted from alfalfa root callus in the mist chamber was adjusted to provide 5000 nematodes/50 ml for inoculation. 1 month after transplanting, the plants were inoculated with the *R. citrophilus*. Irrigation was avoided for 1 day after inoculation to enable greater nematode infection of the plants.

Leaf damage was recorded weekly. During the experiment, plants were treated with 0.51 mg metalaxyl/pot and drenched with 50 ml of 2250 µg iprodione/l to control damping off. Five months after inoculation, the cholorophyll content of the youngest fully mature

leaves was measured using a chlorophyll meter SPA D-502 (Minolta Co., Ltd., Japan) and the plant fresh and dry weights, longest root lengths, and root damage (black root number divided by total root number) were recorded. Nematodes were then extracted from media and tissues in a mist chamber over 5 days.

ANOVA was used to analyze parametric variables and nonparametric (ranking sequence) variables. Parametric variables refers to relative dry weight, relative weight gain (difference between final fresh weight and initial fresh weight), relative root damage, relative new leaves to total leaves ratio, relative longest root length, relative SI, relative leaf retention, relative chlorophyll content, and nematode reproduction [log(Rf+1)]; nonparametric variables refers to the ranking of the corresponding variables. Relative refers to the difference between inoculated and uninoculated treatments. SI and leaf retention were calculated as for in vitro screening. The Waller-Duncan's multiple-range test was used to compare differences among cultivars and treatment means. Correlation of each parameter between in vitro and ex vitro tests was conducted.

3.4. Results

In vitro screening

Tolerance among *Anthurium* cultivars was differentiated by use of the nonparametric variable, ranking of relative leaf retention (P=0.02) but not by ranking of symptom index (P=0.11). Evaluation of tolerance with the parametric variables, symptom index difference and relative leaf retention, showed high variation within cultivars (P=0.0005 and P=0.0065 respectively). A lower ranking of leaf retention resulted from a smaller difference between inoculated and uninoculated treatments, and thus represented a relatively tolerant cultivar. Therefore, 'Blushing Bride' was ranked as the most tolerant cultivar (lowest ranking of relative leaf retention; Fig. 3.1).

When actual leaf retention among inoculated plants was compared, 'Midori' rated the highest (P=0.015; Fig. 3.2), but variation within cultivars was high (P<0.01). Both the inoculated and uninoculated leaf retention for 'Midori' was greater than those cultivars in the tolerant and susceptible group, i.e., 'Anuenue', 'Blushing Bride' or 'Marian Seefurth' (Fig. 3.2). This showed that 'Midori' was more vigorous. The performance of 'Midori' was considered to be more stable in different environments as interpreted by its lower standard deviation (Fig. 3.2).

Resistance among *Anthurium* cultivars was also differentiated better by use of a nonparametric variable, namely the ranking of nematode reproduction (P=0.0015). Differences were less significant when analyzed by the parametric variable, nematode reproduction (P=0.1). 'Nitta' had the highest ranking of nematode reproduction and thus represented the most susceptible of the cultivars (Fig. 3.3).

Relative leaf retention was correlated with relative symptom index (R^2=0.88, P=0.0001). However, there was no correlation between ranking of Rf and relative leaf retention (R^2=0.02, P=0.85) or relative SI (R^2=-0.06, P=0.62).

Clustering the 17 *Anthurium* cultivars into one of four groups placed 'Ozaki', 'Kalapana', 'ARCS Hawaii' and UH1181 together. These cultivars had a relatively high ranking in relative leaf retention and a low ranking in nematode reproduction, thus representing an intolerant but resistant group.

'Kozohara', 'Alii', 'Tropic Flame', 'Tropic Mist', 'Flamingo Blush', 'Mauna Kea', 'Hawaiian Butterfly' and 'Midori' grouped together because of moderate ranking of relative leaf retention and moderate ranking of nematode reproduction. Though the tolerance measurement of these cultivars was not different from the previous group, according to a Waller-Duncan k-ratio t-test, their tolerance might be higher as the nematode population was greater. Therefore, this group was considered moderately tolerant but susceptible.

'Nitta' and 'Fujii Pink' formed a third group having high relative leaf retention and high nematode reproduction. These two cultivars had a higher nematode reproduction than any other cultivar ($P<0.02$; Fig. 3.2), and therefore should be considered as relatively intolerant and susceptible cultivars.

'Anuenue', 'Marian Seefurth', and 'Blushing Bride' comprised the fourth group with low rankings in relative leaf retention and a moderate nematode reproduction. This group should be considered as tolerant and susceptible.

Ex vitro Screening

Cultivar tolerance can be differentiated by relative weight gain ($P=0.0004$) and relative leaf retention ($P=0.08$). Cultivar tolerance can also be differentiated by nonparametric variables, including ranking of relative dry weight ($P=0.08$), and ranking of relative weight gain (p=0.009). Correlations between in vitro and ex vitro results were found. Relative leaf retention in the in vitro screening was correlated with the relative weight gain (correlation coefficient=0.59, $P=0.09$), and relative root length (correlation coefficient=0.6, $P=0.09$) in the ex vitro screening. The standard intolerant cultivar identified in vitro, i.e., 'Ozaki', also showed relatively low *R. citrophilus* tolerance ex vitro as observed from its high relative weight gain and high relative leaf retention (Fig. 3.4.). 'Midori', had relatively high tolerance as shown by its low relative leaf retention and high relative weight gain. 'Blushing Bride', however, did not tolerate *R. citrophilus* under ex vitro conditions; it had higher relative leaf retention. Thus, *R. citrophilus* tolerance of some cultivars deviated from the in vitro results. These cultivars included 'Tropic Mist' and 'Fujii Pink', which showed higher tolerance than 'Midori' (Fig. 3.4.).

Nematode resistance was unable to be determined in this ex vitro trial as most of the cultivars had zero nematode extracted. Means of nematodes extracted from the tissue among the cultivars ranged from 0 to 0.17 and nematodes extracted from the growing medium were also 0, with the exception of 106 nematodes extracting from 'Fujii Pink'.

3.5. Discussion

Screening Methods

Lack of correlation between Rf and leaf retention or between Rf and SI confirmed that tolerance and resistance are independent traits in *Anthurium* as suggested by Trudgill (1985). Thus screening parameters should be evaluated separately. No complete tolerance and resistance was found among the 17 *Anthurium* cultivars evaluated in vitro because none of the cultivars had relative leaf retention nor Rf values less than 0 or less than 1, respectively. A bias may arise from using a 3-month trial for resistance screening, because nematode populations may crash after plant tissue is highly damaged. Consequently, late measurements of resistance parameters may show lower populations than would have otherwise been observed at an earlier evaluation. To increase the efficiency of screening, future selection for *R. citrophilus* resistance and tolerance should be conducted separately. In vitro screening for nematode resistance in peach scion cultivars to *M. incognita* was conducted only for 5 weeks (Huettel and Hammerschlag, 1993). Hence future screening for *R. citrophilus* resistance in *Anthurium* might need to be shortened from 3 months to a period before the test materials become highly damaged by nematode infection.

Use of nonparametric variables was proved to be a more reliable method for nematode tolerance and resistance evaluation in this in vitro screening experiment. Ranking of variable was previously recommended by Trudgill (1985) for resistance evaluation. In our study, ranking of relative leaf retention is recommended for tolerance evaluation. Nevertheless, although relative leaf retention represents plant tolerance with respect to the uninoculated control, it does not represent plant vigor. For example, in the in vitro screening, 'Midori' is less tolerant than 'Blushing Bride' in terms of ranking of relative leaf retention, but it is actually more vigorous than 'Blushing Bride' as indicated by its higher

leaf retention under inoculated and uninoculated conditions. Therefore, leaf retention value of the inoculated plants should not be ignored in selection for tolerance as it reflects the actual plant vigor which, in turn, may affect overall plant productivity.

Reference for Breeding Tolerant and Resistant Varieties

Based on the in vitro screening results, several cultivars can be selected as references for future evaluations. 'Ozaki', a red heart shaped *Anthurium*, reacted poorly to *R. citrophilus* infection and can represent an intolerant reference. 'Nitta', an orange *Anthurium* cultivar, supports the highest nematode population density and thus represent a susceptible reference. No resistant cultivar was identified among the tested cultivars. Though 'Blushing Bride' was identified as the most tolerant cultivar, it may not be an ideal tolerant reference due to its low vigor.

Genetic Background of Tolerant Cultivars

'Anuenue' and 'Marian Seefurth', which are scored as tolerant in vitro, had the same parents, 'Haga'×Pink clone (Acc11; Appendix A). 'Haga' is an obsolete cultivar and not available for screening. Many of the other cultivars ('Alii', 'ARCS Hawaii', 'Blushing Bride' and 'Kalapana') also have 'Haga' in their pedigree. However, as the cultivar's co-ancestry with 'Haga' decreases, tolerance capability also decreases except 'Blushing Bride' which was identified to be tolerant. It is most probable that tolerance genes contributed from 'Haga' have low heritability. Tolerance might also be contributed from genes associated with vigorous growth, as might exist in 'Midori'. Unfortunately the parents of 'Midori' are unknown.

Consistency of Results for In vitro and Ex vitro Screening

Correlation between in vitro and ex vitro screening results for *R. citrophilus* tolerance in *Anthurium* confirmed the reliability of the in vitro screening system. The reliability of in vitro screening for nematode resistance or tolerance was previously been demostrated in peach with *M. incognita* (Huettel and Hammerschlag, 1986; 1993); in

soybean for *Heterodera glycines* (Lauritis et al., 1982); in tomato for root-knot nematode resistance (Fassuliotis and Bhatt, 1982); and in grape for lesion nematode, *Pratylenchus vulnus*, tolerance (Palys and Meredith, 1984).

Among the parameters used in the ex vitro screening, differences between cultivars can only be differentiated by the parametric and nonparametric variables of relative weight gain and relative leaf retention. This is because relative weight gain and relative leaf retention were adjusted to the initial plant weight. These adjustment overcome the high variation generally observed in the ex vitro screening.

However, this ex vitro screening trial failed to identify *R. citrophilus* resistance due to a nematode population crash during the screening period. Screening for tolerance in this trial was reliable as shown from the difference obtained between inoculated and uninoculated treatments and the correlation of the results to in vitro results. The nematode population crash might be caused by an adverse environment (49°C, 20%RH) recorded at least once in the greenhouse during the screening period. This environmental effect could be avoided under proper in vitro screening conditions.

In vitro screening results were partially consistent with anecdotal *Anthurium* field observations as listed in Table 3.1. 'Ozaki' was identified as intolerant in both field observations and in vitro screening. 'Midori' was evaluated as having poor performance in the field, in contrast to the tolerant performance evaluation it received in vitro. One explanation for this inconsistency may be a nonuniform nematode distribution in the field. Moreover, since 'Midori' is evaluated as a tolerant but susceptible cultivar during in vitro screening, the nematode number in 'Midori' might have become too high for the plant to tolerate during the grower evaluation. This is one of the disadvantages of tolerance.

Reference Index for Burrowing Nematode Tolerance and Resistance Screening

The standard susceptible or intolerant cultivar can serve as an internal control and check for inoculum effectiveness. In soybean cyst nematode resistance breeding, 'Lee' is

commonly used as standard susceptible cultivar and an index of nematode parasitism is based on the average number of females on a differential divided by the average number of females on 'Lee' (Schmitt and Shannon, 1992). Expressing the number of nematodes developing in partially resistant genotypes as a percentage of that on a susceptible control can also be used to quantify resistance (Trudgill, 1985). Phillips and Trudgill (1983) suggested that when testing unknown genotypes, cultivars of known resistance must be used as internal standards to delineate resistance categories. Standard tolerant and resistant cultivars also are important as they can provide a standard level of selection. The high leaf retention of 'Midori' under in vitro screening condition and the high tolerant performance of 'Midori' under ex vitro screening condition confirmed that 'Midori' should be the standard tolerant cultivar. A standard resistant cultivar requires further evaluation to identify.

Due to the high variation in tolerance measurement observed with in vitro screening, ranking sequence is recommended for *R. citrophilus* tolerance selection in *Anthurium*. In this regard, use of a standard intolerant cultivar as a selection reference would not give a reliable index. Direct selection of *Anthurium* progenies that perform better than 'Midori' or 'Blushing Bride' under the same inoculation condition would be a better reference. Since no resistant cultivar can be identified, future selection for *R. citrophilus* resistance should rely on a resistance index obtained by the log(Rf of the progeny tested+1) divided by log(Rf of 'Nitta'+1) under the same environment.

Suggestion for Future Burrowing Nematode Control in *Anthurium*

Selection towards high tolerance with moderate resistance to *R. citrophilus* is a desirable approach. This will avoid selection pressure on nematode virulence if highly resistant cultivars were planted. 'Midori' and 'Blushing Bride', may be desirable cultivars to use as parents. In addition to this strategy, *Anthurium* growers may also want to practice mixing culture of tolerant and resistant cultivars in a single planting. It has been

demonstrated that the increase in soybean nematode reproduction ability on resistant cultivars was higher than in the continuously planted rotation of resistant with susceptible cultivars (Young and Hartwig, 1992). However, the continuously cropped resistant cultivar had a higher yield than the continuously cropped susceptible soybean. Therefore, if a rotation of resistant with tolerant cultivars is practiced, nematode number in the field could be controlled while plant yield could be improved by the tolerant cultivars. Since *Anthurium* is a longer term crop (repropagated every 5 or more years; Higaki et al., 1994), the rotation period might be too long for this cultural practice to be effective. A new alternative method to control *R. citrophilus* in *Anthurium* would be to grow both resistant and tolerant cultivars in the same field.

One unanswered question in this screening method is the consistency in performance between juvenile, seedling-sized plants and mature plants. Canals and Pinochet (1992) showed that peach-almond hybrid expressed resistance in 1-year-old plants but not 1-month-old plants. Screening of the mature *Anthurium* plants for burrowing nematode resistance and tolerance might further confirm our screening reliability.

Table 3.1. *Anthurium* cultivars chosen for in vitro screening and their *Radopholus citrophilus* susceptibility as evaluated by a grower in the field.

Cultivar	Susceptibility	Cultivar	Susceptibility
Marian Seefurth		Mauna Kea	R
Kozohara		Kaumana	
Ozaki	S[z]	Kalapana	R
Hawaiian Butterfly			
Paradise Pink		(New cultivars)	
Anuenue	M	Alii	S
Nitta Orange	M	Blushing Bride	M
Flamingo	R	Tropic Flame	M
Midori	S	UH1181	
Fujii Pink			

[z]S=susceptible, M=moderate resistant, R=resistant. Rating not available for all cultivars.

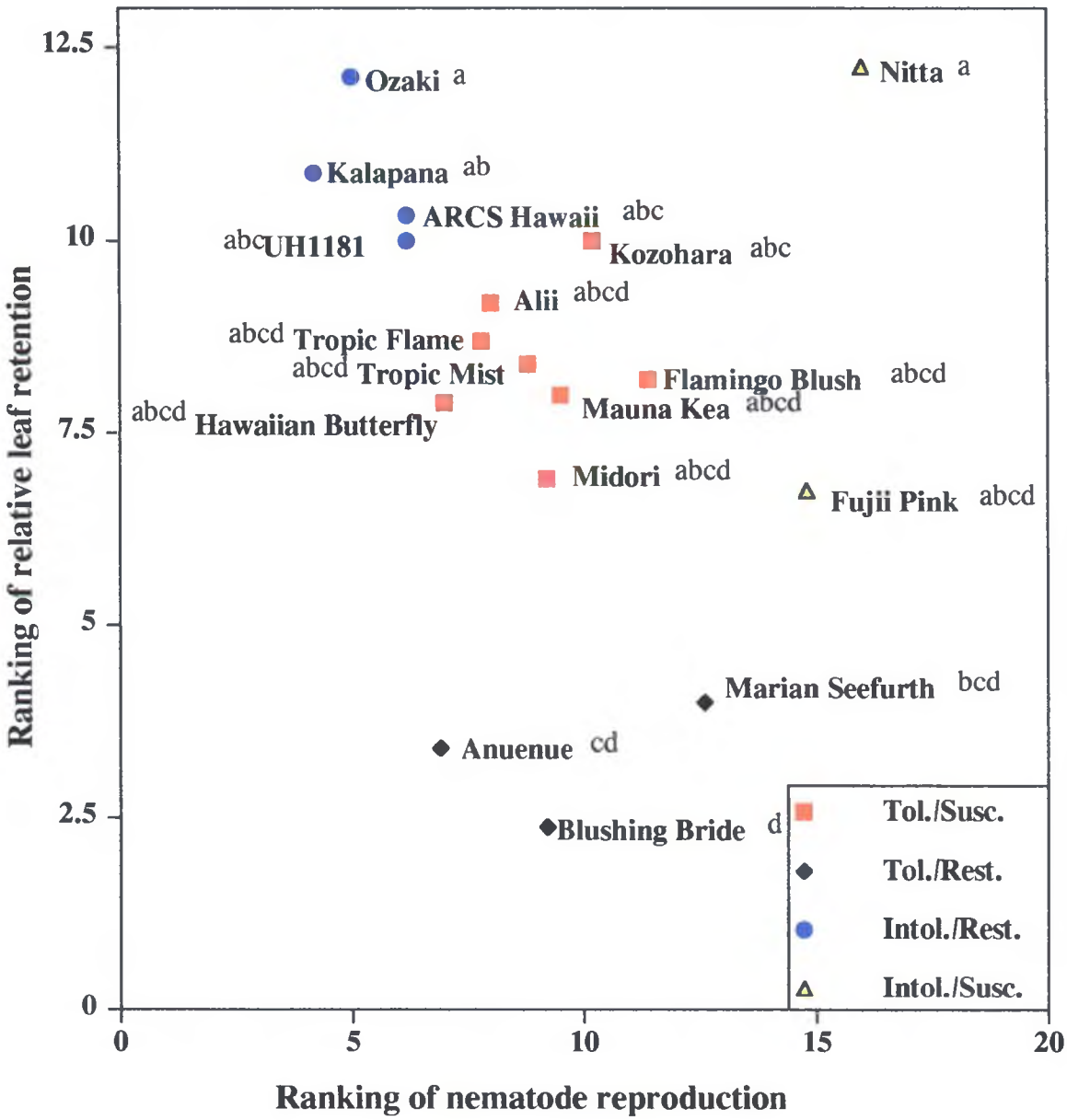

Fig. 3.1. Plot of ranking of relative leaf retention by ranking of nematode reproductive factor (Rf) for 17 *Anthurium* cultivars screened in vitro 3 months after inoculation with *Radopholus citrophilus*. Data points with the same color are clustered according to the Fastclus procedure (SAS). Leaf retention = % green leaves remaining per total leaves. Relative refers to the difference between inoculated and uninoculated plants. Cultivars followed by the same letters do not differ in leaf retention according to the Waller-Duncan k-ratio t-test.

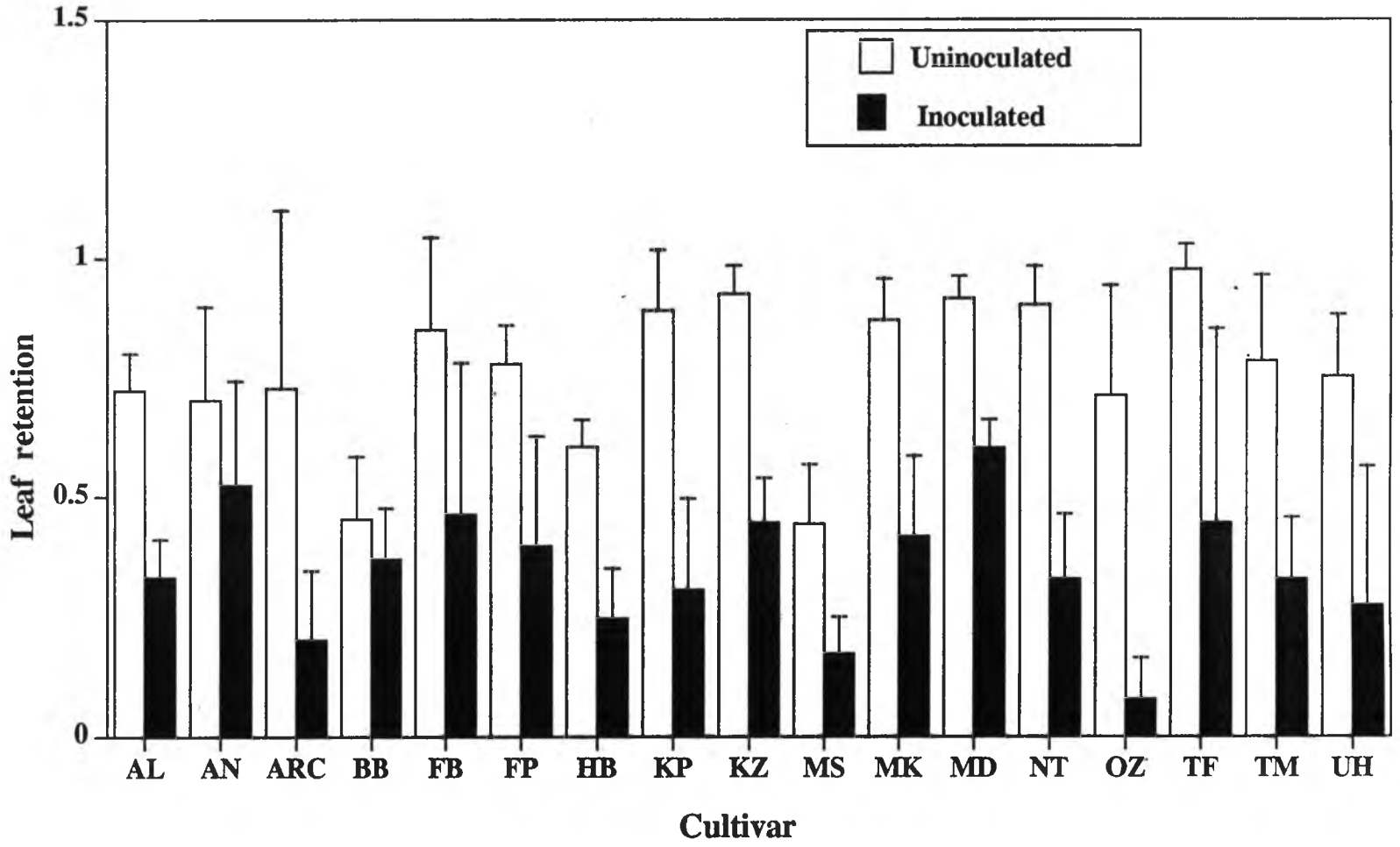

Fig.3.2. Leaf retention of inoculated and uninoculated plants of *Anthurium* cultivars. AL= 'Alii', AN= 'Anuenue'; ARC= 'ARCS Hawaii'; BB= 'Blushing Bride'; FB= 'Flamingo Blush'; FP='Fujii Pink'; HB= 'Hawaiian Butterfly'; KP= 'Kalapana'; KZ= ' Kozohara'; MS= 'Marian Seefurth'; MK= 'Mauna Kea'; MD= ' Midori'; NT= 'Nitta'; OZ= 'Ozaki'; TF= 'Tropic Flame'; TM= 'Tropic Mist'; UH=UH1181.

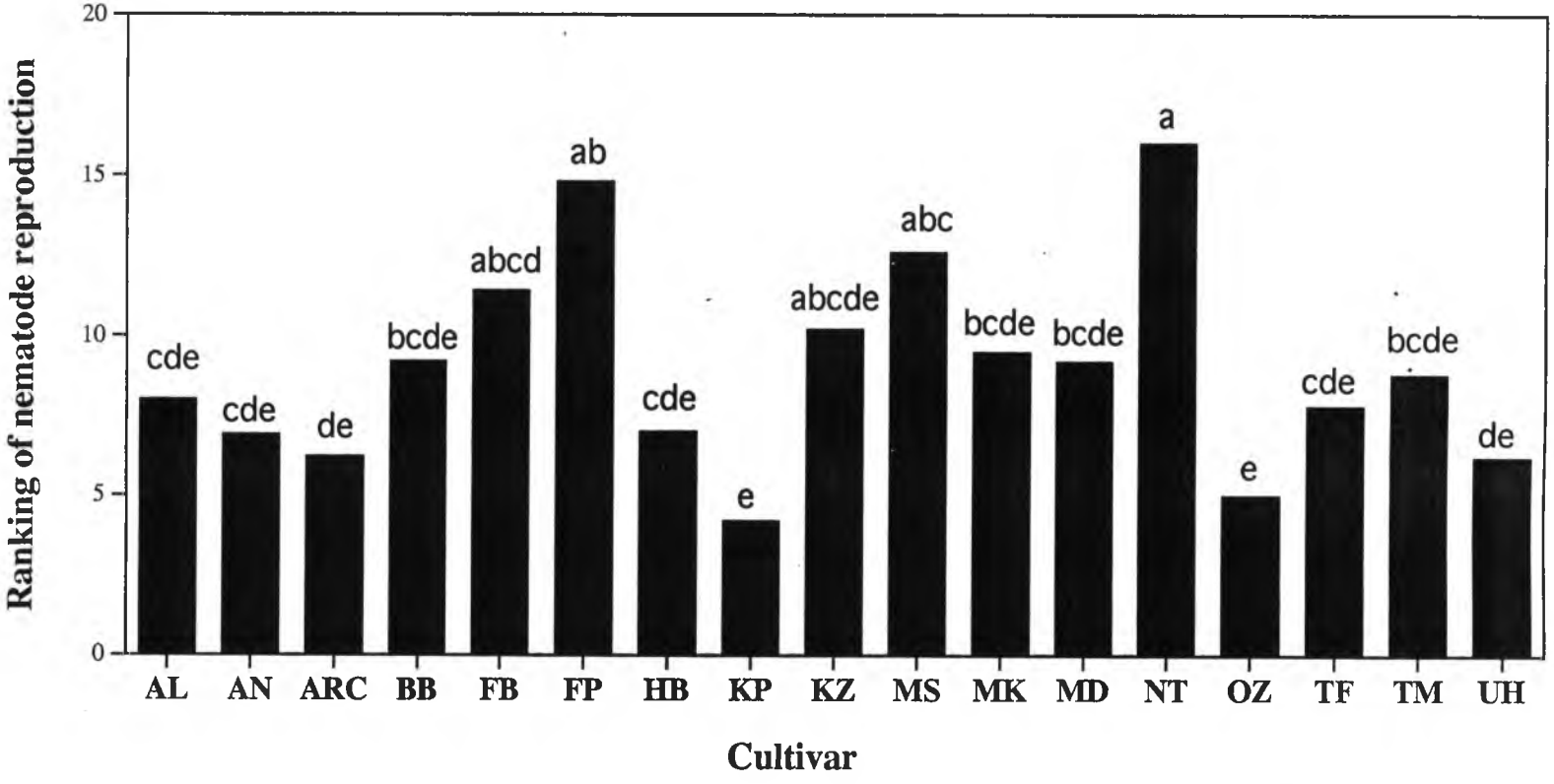

Fig. 3.3. Nematode reproduction, log(Rf+1), in *Anthurium* root tissue 3 months after in vitro screening. AL= 'Alii', AN= 'Anuenue'; ARC= 'ARCS Hawaii'; BB= 'Blushing Bride'; FB= 'Flamingo Blush'; FP='Fujii Pink'; HB= 'Hawaiian Butterfly'; KP= 'Kalapana'; KZ= ' Kozohara'; MS= 'Marian Seefurth'; MK= 'Mauna Kea'; MD= 'Midori'; NT= 'Nitta'; OZ= 'Ozaki'; TF= 'Tropic Flame'; TM= 'Tropic Mist'; UH=UH1181. Rf=Final nematode population divided by initial nematode population. Cultivars followed by the same letters do not differ in Rf according to Waller-Duncan k ratio t-test (k=100).

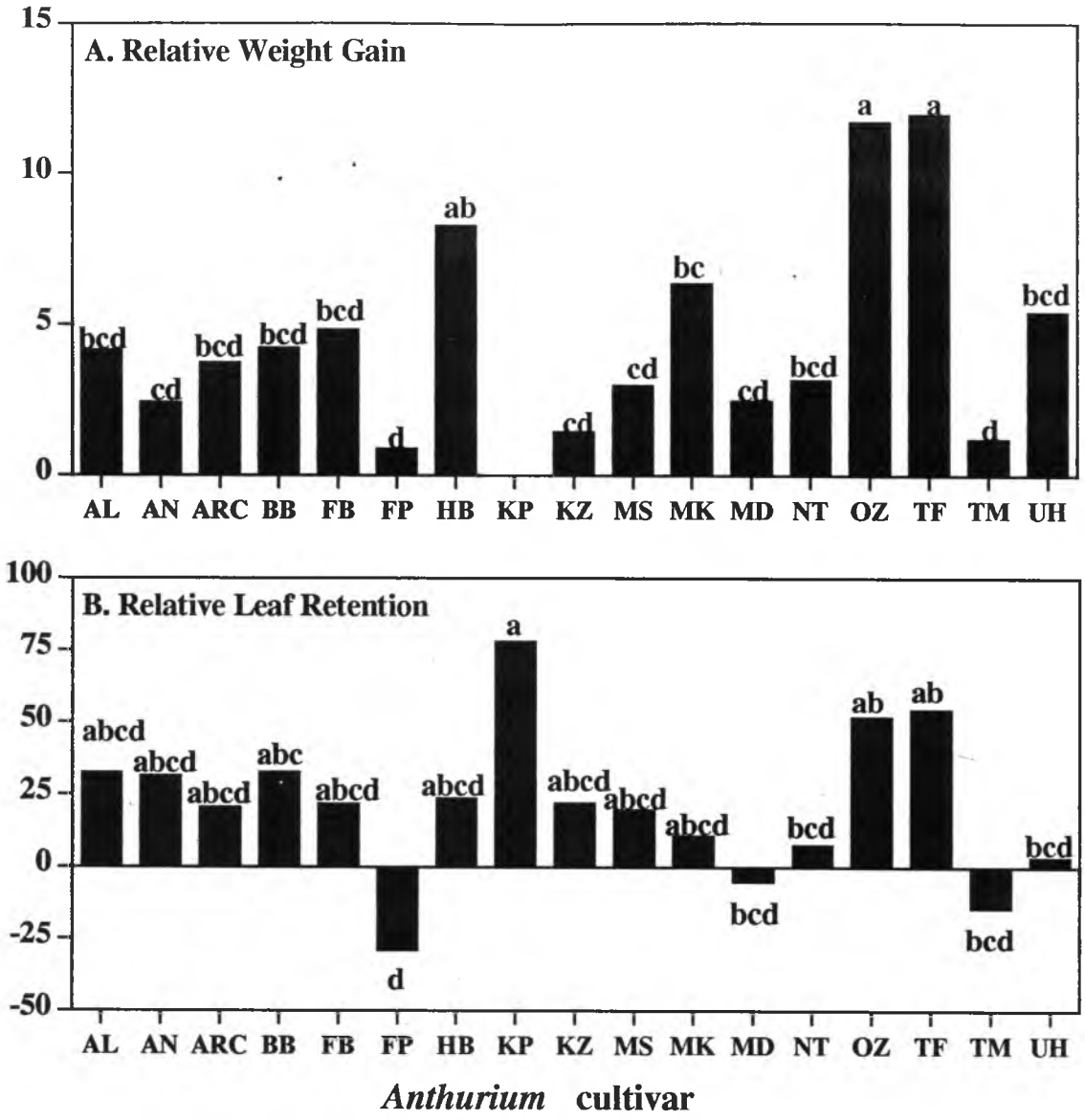

Fig. 3.4. (A) Relative weight gain and (B) Relative leaf retention of *Anthurium* cultivars, AL= 'Alii', AN= 'Anuenue'; ARC= 'ARCS Hawaii'; BB= 'Blushing Bride'; FB= 'Flamingo Blush'; FP='Fujii Pink'; HB= 'Hawaiian Butterfly'; KP= 'Kalapana'; KZ= ' Kozohara'; MS= 'Marian Seefurth'; MK= 'Mauna Kea'; MD= 'Midori'; NT= 'Nitta'; OZ= 'Ozaki'; TF= 'Tropic Flame'; TM= 'Tropic Mist'; and UH=UH1181, 5 months after *Radopholus citrophilus* inoculation in the greenhouse. Relative refers to difference between inoculated and uninoculated plants. Leaf retention is the percentage of green leave to total leave number. Cultivars followed by the same letters do not differ according to Waller-Duncan k ratio t-test (k=100).

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Chapter 4. Distribution of Citrus Burrowing Nematode in *Anthurium*

4.1. Abstract

Anthurium andraeanum 'Alii' and 'Midori' plants inoculated in the greenhouse with *Radopholus citrophilus* were evaluated for the presence of nematodes in the shoots, roots, and petiole of the lowest leaf. *R. citrophilus* were found in all tissues in numbers ranging from 1 to 88 in shoot sections, 26 to 67 in roots, and 1 to 165 in petioles of both cultivars. Nematode invasion was highest in the roots of 'Midori' and in the basal stem section of 'Alii'. There might be a relationship between nematode distribution in tissues and plant tolerance to nematodes. Propagation by stem cuttings cannot guarantee nematode-free planting material.

4.2. Introduction

Burrowing nematodes, *Radopholus citrophilus*, are common pathogens of many tropical plants (Holtzmann et al., 1984). Most migratory endoparasitic nematodes are found predominantly in root tissue. Recently, a propagule of *Anthurium* stem taken 30.5 cm above the growth medium was found to be infected with burrowing nematode, even though its subtending aerial roots and the cinder medium were free of nematodes (Goo, 1995). As apical stem cuttings (top cuttings) are a common *Anthurium* propagule (Higaki et al., 1994), and *R. citrophilus* is known to enter the stele of citrus roots (DuCharme, 1959), it becomes of interest to determine if this nematode routinely invades shoot tissue in *Anthurium*. The objectives of this study were to examine the frequency and extent of nematode invasion of *Anthurium* tissues and to test for cultivar differences.

4.3. Materials and Methods

Anthurium andraeanum 'Alii' and 'Midori', planted in 3.8 liter black pots filled with 1:1 (v/v) Big R pine bark compost-perlite; cinder; 2:1 (v/v) cinder-peat; or 1:2:1 (v/v/v) rockwool (1 part water resistant and 1 part water absorbent)-cinder-peat moss were inoculated with 2000 *R. citrophilus,* were evaluated for nematodes in the shoot tissue. The experiment was conducted in a random complete block design with six replications. Nematode inoculum was obtained from alfalfa root callus maintained in vitro (Ko et al., 1996). After 9 months, twelve plants were collected from each cultivar with equal samples from each medium.

Nematodes were extracted for 5 days from three sections of the stem, the petiole of the first leaf from the base, and total roots using a mist chamber. Stem tissue located 0-3 cm, 3-6 cm, and beyond 6 cm from the base were extracted individually for nematodes. For petioles, one sample from each treatment (cv × medium) was chosen and nematodes were extracted from the entire petiole of the lowest leaf.

General linear model analysis of means of 12 samples from each cultivar were conducted to compare the nematode number per gram weight of tissue between cultivars and between plant tissue. Duncan's multiple-range test was used to compare differences among plant tissues. The frequency of plants with nematodes was calculated for each tissue, and a 2×2 χ_1^2 (chi-square) test was used to analyzed the difference between cultivars.

4.4. Results

Burrowing nematodes were found in all shoot sections, leaf petioles and root tissue in both 'Alii' and 'Midori' (Table 4.1). Nematode number did not differ between cultivars nor among tissues ($p>0.05$; Table 4.1) except in the root. 'Midori' had higher nematode numbers in the roots than 'Alii' ($p<0.05$). In 'Midori', nematode number was higher in the

root as compared to the other tissues ($P<0.05$; Table 4.1). Nematodes accumulated in the 0-3 cm shoot section and in the petiole in 'Alii' ($P>0.05$, Table 4.1). However, some stems contained nematodes in the second section (3-6 cm) without any nematodes in the lower section.

Frequency of nematode invasion in shoot sections of 3-6 cm and beyond 6 cm was greater in 'Midori' than in 'Alii', whereas those in petioles were higher in 'Alii' than 'Midori' (Table 4.2.)

4.5. Discussion

Burrowing nematodes are not limited to *Anthurium* root tissue, but are able to migrate from root tissue into stem and even petiole tissues. Studies of morphogenesis and histopathology of citrus roots infected by *Radopholus similis* showed that nematodes entered growing root tips in the region of elongation and root hair production and invaded successive cells by lysis (DuCharme, 1959). All types of parenchyma tissues were attacked, except suberized epidermis, exodermis, endodermis, mature xylem, hardened pith, peridermis, and hardened root cap. Nematodes penetrated through the passage cells of the endodermis to destroy the phloem-cambium ring. Burrowing nematode thus can penetrate through the endodermis of the younger roots where endodermis has not been suberized, and invade into the stelar region. Therefore, it was not surprising that citrus burrowing nematode can invade shoot tissue and migrate in the shoot tissue of *Anthurium*.

Burrowing nematode has not been reported in the stelar region of other hosts such as palms, avocado, and banana (Koshy et al., 1991; Jasy and Koshy, 1992; Holdeman, 1986). In banana, penetration of burrowing nematode into the endodermis of roots was due to coinfection with *Fusarium oxysporum* (Holdeman, 1987). Similar coinfection was not observed in *Anthurium*. As lesion nematodes have been found in the stem and leaves of agloanema (D. Schmitt, UH Department of Plant Pathology, personal communication),

it is possible that members of the aroid family have relatively less suberized or hardened tissue that could lead to the penetration of nematode into the stele.

The higher nematode invasion in the roots of 'Midori' and in the basal stem section of 'Alii' suggested that there might be a relationship between nematode distribution in tissues and nematode tolerance. Previous results (Chapter 3) identified 'Midori' as more tolerant to burrowing nematode than 'Alii'. The restriction of nematode reproduction to the root tissue in 'Midori' might explain one possible tolerance mechanism in *Anthurium*. Greater frequency of nematode invasion in the leaf petiole in 'Alii' might explain the lower leaf retention (index for tolerance) associated with this cultivar.

In conclusion, this information suggests that propagation by stem cuttings cannot guarantee nematode-free planting material. There is less risk of burrowing nematode invasion into the shoot of the tolerant cultivar, 'Midori', than into 'Alii'.

Table 4.1. Number of *Radopholus citrophilus* per g-weight tissue in shoot sections (0 to 3; 3 to 6; and more than 6 cm from the base), petiole of the first leaf from the base, and root tissue in two *Anthurium* cultivars 9 months after inoculation.

Tissue	'Alii'	'Midori'	MSE(cultivar)
Stem Sections from the base			
0-3 cm	88 a	7 b	76.26
3-6 cm	3 a	3 b	3.86
>6 cm	1 a	0.3 b	1.44
First petiole from the base	165 a	1 b	201.89
root	26 a	67 a	12.76
MSE(tissue)	106.15	28.77	

Data are the means of 8 replications. Means followed by the same letter in the same column are not different within cultivar ($P<0.05$), according to the Waller-Duncan k-ratio t-test (k=100).

MSE=Mean square error (between the cultivars or the tissue sections).

Table 4.2. 2×2 χ_1^2 analysis for frequency of nematode invasion.

tissue	2×2 χ_1^2	P
Stem Sections from the base		
0-3 cm	1.29	0.250
3-6 cm	5.95	0.025 *
>6 cm	74.26	0.005**
First petiole from the base	5.13	0.025**

* and ** indicate significant differences at $P \leq 0.05$ and $P \leq 0.01$, respectively.

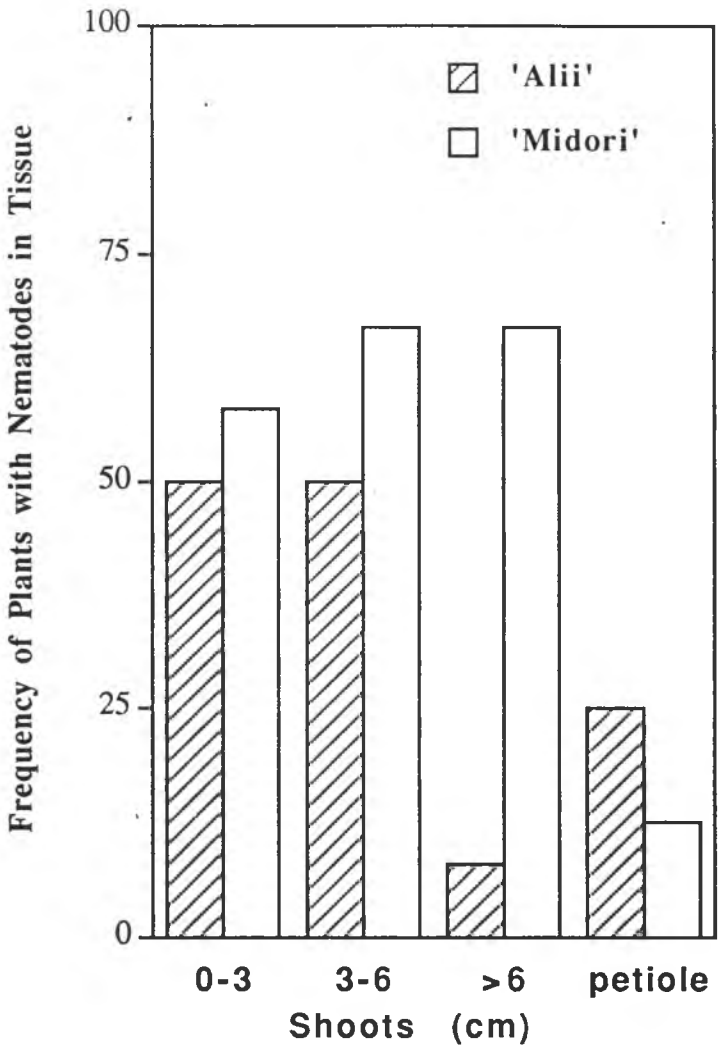

Fig. 4.1. Frequency of plants with nematodes in 3 shoot sections and the petiole of the first leaf from the base in *Anthurium andraeanum* 'Alii' and 'Midori' 9 months after inoculation with *Radopholus citrophilus* . Data are frequency of 12 replications in shoot sections and 8 replications for the petiole.

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Chapter 5. Relationship between Burrowing Nematode, Host-Resistance and Tolerance, and Plant Nutrient Content in *Anthurium*

5.1. Abstract

Six *Anthurium* cultivars previously evaluated in vitro as tolerant, intolerant, and moderately tolerant to *Radopholus citrophilus* were analyzed for nutrient content and the nutrient content was correlated with their tolerance (weight gain, leaf retention, symptom index, root damage) and resistance (nematode reproduction in plant tissue) measurements. Tolerance to nematodes was negatively correlated with Fe, Cu, and Ca content and the (Ca+Mg)/K ratio whereas resistance was negatively correlated with the (Ca+Mg)/K ratio and positively correlated with K content. No correlation of nematode resistance or tolerance to N, P, Na, Mn, or Zn was found. (Ca+Mg)/K is proposed as a marker for selection of *Radopholus citrophilus* tolerance and resistance in *Anthurium*.

5.2. Introduction

Burrowing nematode, *Radopholus citrophilus*, causes poor vegetative growth and decreases flower yield of *Anthurium*. Infected plants have necrotic and chlorotic leaves, and roots with lesions (Aragaki and Apt, 1984). These symptoms are characteristic of nutrient deficiency.

Contradictory reports concerning the impact of nematodes on host plant nutrient content exist. Tomato infected with *Meloidogyne incognita* had slightly greater P content (Oteifa et al., 1958), and oats infected with *Heterodera avenae* had increased K and P uptake rates compare to uninfected controls (Price et al., 1982). Similar results were observed in a study of soybean cyst nematode-infected soybean. Root concentration of K and Mg decreased with concurrent increase in Ca and P, and leaf concentration of Mg and Ca increased (Blevins et al., 1995). However, Trudgill et al. (1975) showed that potato

infected with potato cyst nematodes contained less P and K and more Ca than uninfected plants.

No reported studies describe the effects of burrowing nematode infection on plant nutrient content in *Anthurium*. The objectives of this study were to determine the correlation of nutrient content to burrowing nematode resistance or tolerance in *Anthurium*, and to understand if tolerance or resistance is related to water use efficiency and nutrient availability.

5.3. Materials and Methods

An in vitro screening trial was established to test the relationship between nutrient content of *Anthurium* cultivars and their tolerance and resistance to *R. citrophilus*. Six cultivars; 'ARCS Hawaii', 'Blushing Bride', 'Mauna Kea', 'Midori', 'Ozaki' and 'Tropic Mist', previously evaluated (Chapter 3) as relatively tolerant, intolerant, susceptible or moderately tolerant, were tested. Each experimental unit consisted of four 3 cm-tall plantlets, with total weight (of four 4 plants) of about 1 g, established for 1 month in a GA-7 box (Sigma, St. Louis, Mo). Before inoculation, 1 g of plant tissue from a mix of leaves of the four plants in each box was collected and oven dried at 70°C for 24 hours for nutrient element analysis. Nematode inoculum was obtained from alfalfa root callus culture as described previously (Ko et al., 1996). Nematode number was estimated by alfalfa root length. Four hundred nematodes were inoculated into each box. Inoculated treatments were arranged pairwise in a random complete block design with four replicates repeated over four different times.

Analysis of 11 plant essential elements (N, P, K, Ca, Mg, Na, Mn, Fe, Cu, and Zn) was conducted by the Agricultural Diagnostic Service Center of the University of Hawaii at Manoa. Four replicates were tested for N and Ca content whereas 2 replicates were tested for the other elements. Inoculated and uninoculated plants were used to

obtained a symptom index and to establish differences in leaf retention (see Chapter 3), root damage (black root to total root ratios), and weight gain (difference between final fresh weight and initial fresh weight) for each cultivar.

Nematodes were extracted from the media and from plant tissues in a mist chamber. Nematode reproduction, Rf (final nematode number/ initial nematode number), in root and medium were both transformed by log (Rf+1) to reduce variance. Nutrient content of N, P, K, Ca, Mg and Na was measured in percentage whereas Mn, Fe, Cu and Zn contents were measured in μg/g. Analysis of variance was performed on the preinoculation nutrient content of those plants in the inoculated treatment and means were compared by Duncan's multiple-range test. Data were further analyzed by correlation and regression between tolerance and resistance measurements and nutrient content of the 11 elements evaluated.

5.4. Results

Correlation analysis showed that weight gain of *Anthurium* was negatively correlated with Fe, Cu, Ca and (Ca+Mg)/K content (Table 5.1). Rf in the tissue was positively correlated with (Ca+Mg)/K (R^2=0.67, P=0.03) and Rf in the medium was negatively correlated with K (R^2=-0.59, P=0.06; Table 5.1).

Cu, K/Ca and (Ca+Mg)/K differed among the cultivars (P<0.05; Table 5.2). Among the tolerant cultivars, 'Blushing Bride', 'Mauna Kea', and 'Midori' had relatively high K/Ca ratios but lower (Ca+Mg)/K ratios. The moderately tolerant cultivar, 'Tropic Mist', had the lowest level of Cu. No correlation of nematode resistance or tolerance to N, P, Na, Mn, or Zn was found. Regression between weight gain and (Ca+Mg)/K ratio was given by the equation:

Weight gain = 17.26 – 92.15 (Ca+Mg)/K (R^2=0.6445, P=0.0052).

5.5. Discussion

Nutrient analysis results indicated that tolerance to *R. citrophilus* in *Anthurium*, as measured by plant weight gain, is related to a decreased Ca accumulation, as seen also for potato infected by cyst nematodes (Evans and Franco, 1979; Trudgill and Cotes, 1983). Furthermore, tolerance was negatively correlated with (Ca+Mg)/K but positively correlated with K/Ca, consistent with results for potato cyst nematode tolerant cultivars (Evans and Franco, 1979). Differences in cation accumulation, their ratios, and in plant weight gain among cultivars may relate to their water use efficiency (Evans and Franco, 1979). A study of cation relations by Reinbott and Blevins (1991), showed that Ca and Mg uptake and translocation mechanisms, i.e., passive uptake and active efflux, are similar under the same P treatments. K depresses the translocation of Mg and Ca from roots to shoots. With increasing P, K decreases, and Mg and Ca concentration increase, resulting in a decreased in K/(Ca+Mg) as seen in *Anthurium*.

The (Ca+Mg)/K ratio is also negatively correlated with resistance of various crops to root-knot nematode (Bains et al., 1984). This results support such a relationship in *R. citrophilus* resistance in *Anthurium*. Furthermore, the negative correlation between K and nematode reproduction in *Anthurium* was consistent with application of K to decrease the population of the citrus nematode, *Tylenchus semipenetrans*, in roots and soil (Rabeh and Sweelam, 1990). Although resistance and tolerance to nematodes are independent traits (Chapter 3; Trudgill, 1985), the common attribute of low (Ca+Mg)/K ratios is likely due to the interplay of the elements in the plant.

While it has been proposed that P is one of the elements most likely to limit the growth of nematode infected plants (Trudgill, 1980), by increasing nematode resistance or tolerance (Smith and Kaplan, 1988; Martin and Van Gundy, 1963), this study did not validate this. However, the current study presents the first evidence of a correlation of nematode tolerance with Fe and Cu.

Knowledge of relationships between plant nutrition and *R. citrophilus* resistance and tolerance in *Anthurium* will be helpful for future selection of tolerant and resistant cultivars. Selection for indiyidual plants with a low (Ca+Mg)/K ratio would be expected to yield relatively resistant and tolerant cultivars. This selection method would not only shorten the breeding cycle but also eliminate the need to initially inoculate entire populations of *Anthurium* hybrid progenies with nematodes during a screening program. Leaves can be sampled without sacrificing the entire plant during disease screening.

Table 5.1. Correlation between *Radopholus citrophilus* tolerance and resistance measurements and nutrient content in *Anthurium*.

Element	Plant weight gain	Nematode reproduction in tissue, log(Rf[z]+1)	Nematode reproduction in medium, log(Rf+1)
Fe	-0.6403[y] 0.05	ns[x]	ns
Cu	-0.6346 0.0487	ns	ns
(Ca+Mg)/K	-0.8028 0.00520	0.6653 0.0255	ns
K	ns	ns	-0.5890 0.0566
Ca	-0.4678 0.0324	ns	ns

[z]Rf refers to nematode reproductive factor (Rf=Final nematode number/initial nematode number).
[y]The first number in each cell is the correlation coefficient of 12 values followed by the probability (*P*).
[x] Not significant; *P*>0.05.

Table 5.2. Nutrient content of *Anthurium* cultivars before nematode inoculation.

Cultivar	Cu (µg/g)	K/Ca	(Ca+Mg)/K
'ARCS Hawaii'	2085 a	3.65 c	0.39 a
'Blushing Bride'	2045 a	7.76 ab	0.17 c
'Mauna Kea'	1370 abc	9.82 a	0.15 c
'Midori'	1648 ab	6.12 bc	0.22 bc
'Ozaki'	1054 bc	5.93 bc	0.25 b
'Tropic Mist'	638 c	8.58 ab	0.18 bc

Means of each element or element ratio followed by a common letter are not different according to Duncan's multiple range test (n=2; *P*=0.05).

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Chapter 6. *Anthurium* Species Screening for Burrowing Nematode, *Radopholus Citrophilus*, Tolerance and Resistance

6.1. Abstract

Five *Anthurium* species closely related to the two important cultivated commercial *Anthurium*, *A. andraeanum* Lind. ex André and *A. scherzerianum* Schott, and one species of breeding interest were screened in vitro for tolerance and resistance to *Radopholus citrophilus* Huettel, Dickson, and Kaplan, 1984. *A. pittieri* Engl., *A. ravenii* Croat and Baker, *A. antioquiense* Engl. and *A. aripoense* N.E. Br. were more tolerant than the reference standard, 'Midori', based on a lower relative symptom index. *A. antioquiense* and *A. aripoense* had higher root damage than 'Midori' and thus were not considered to be as tolerant as *A. pittieri* and *A. ravenii*. *R. citrophilus* tolerance in these species is mainly due to stronger plant vigor, fewer target roots for nematode infection or higher nematode resistance. *A. ravenii* was among the most resistant species followed by *A. aripoense* and *A. pittieri*. Adjustment of the tolerance measurement to the initial and uninoculated treatment measurement improved the screening method. The slower nematode reproduction in this experiment enabled concurrent screening for tolerance and resistance to *R. citrophilus* in *Anthurium*.

6.2. Introduction

Breeders need a wide genetic base for selection of characters that may be of value for crop improvement. Wild species have played an important role in contributing genes for pest resistance to the cultivated gene pool (Fassuliotis, 1987). The early years of the *Anthurium* improvement program at the University of Hawaii, initiated in 1950, emphasized developing diverse flower color, flower shapes, high yield, and resistance to anthracnose caused by *Colletotrichum gloeosporioides* Cook (Kamemoto, 1981). This

might have narrowed the genetic base from which to select for nematode resistance among cultivars. Fortunately, the genus *Anthurium* is the largest and most diverse in the family Araceae, comprised of about 1000 species (Croat, 1988). These species might provide a good source for citrus burrowing nematode (CBN), *Radopholus citrophilus,* resistant or tolerant germplasm.

Anthurium andraeanum hybrids are the important cut flower crop in Hawaii, while *A. scherzerianum* is popular as a potted plant in Europe. This experiment screened for CBN resistance and tolerance among species in the sections *Calomystrium* (which includes *A. andraeanum*; Engler, 1905) and *Porphyrochitonium* (which includes *A. scherzerianum*). Representatives from two other sections, *Belolonchium*, and *Oxycarpium* were also included in the screening.

6.3. Materials and Methods

Plant materials

Seven *Anthurium* species (Table 6.1) including three *A. andraeanum* Lind. ex André cultivars previously studied for their burrowing nematode tolerance and resistance, were screened in vitro for burrowing nematode tolerance and resistance. Among the cultivars, 'Midori' and 'Mauna Kea' were used as standard tolerant cultivars, whereas 'Ozaki' was used as the standard intolerant cultivar (results from Chapter 3).

Propagation

Open-pollinated seeds of *A. antioquiense* Engl., *A. bicollectivum* Croat, *A. ravenii* Croat & Baker, *A. aripoense* N.E. Br. and *A. pittieri* Engl. were germinated in vitro. Seeds were removed from the berries and disinfected with 10% Clorox plus 1 drop Tween 20 /100 ml for 30 minutes followed by disinfection in 5% Clorox plus 1 drop Tween 20 /100 ml for 30 minutes. The disinfection was carried out on a gyratory shaker. The ovules were moved into sterile conditions under the laminar flow hood, rinsed three times with distilled

sterile water, and plated onto H1 medium (Kuehnle and Sugii, 1991) lacking BA and solidified with 0.3% Gelrite. Ovules were germinated under light at 23°C. Plants subcultured into GA-7 vessels (Sigma, St. Louis, Mo) were 2- to 4-months old before nematode inoculation, depending on the seedling growth rate of the ovules.

'Mauna Kea', 'Midori', 'Ozaki' and *A. scherzerianum* Schott were clonally propagated using established tissue culture plantlets on H1 medium lacking BA. Two weeks before nematode inoculation, *Anthurium* plants were transplanted into GA-7 boxes with four plants per box.

Nematode Inoculation

R. citrophilus collected in 1993 from an *Anthurium* farm on the island of Hawaii by M. Goo (Univ. of Hawaii, Dept. Plant Pathology), labeled as population HA16, was used as the inoculum source. Screening of species was conducted in vitro as in Chapter 3.

Experimental Design and Data Analysis

A random complete block design was used with six replicates repeated over 6 weeks. Initial plant weight, root number, root damage ratio (number of black roots divided by number of total roots), symptom index, and leaf retention (percentage of green leaves retained) were recorded. The symptom index was derived from the summation of percentages of leaf damage levels multiplied by the level of leaf damage. Leaf damage levels were assigned as: Level 0=green, 1=chlorotic, 2=yellow spot, 3=partly yellow, 4=completely yellow, 5=brown spot, 6=partly brown, 7=completely brown. Plants were harvested 3 months after inoculation and final data were recorded. The difference between the final and initial data were calculated. Nematode-inoculated plant tissues and media were placed in a mist chamber for 7 days to extract nematodes. Nematode reproduction data were log transformed by log (Rf+1), where Rf is the final nematode number divided by initial nematode number.

Species or cultivars response to nematode infection was compared by either mean (parametric) or ranking sequence (non-parametric) of weight gain ratio (ratio of weight gain between inoculated and uninoculated), relative root damage, relative gain in root number, relative symptom index, relative leaf retention and nematode reproduction. Relative refers to the difference between inoculated and uninoculated plants. General linear model (GLM) analysis was used to analyze the parametric and nonparametric variables (Eskridge, 1995; Phillips, 1984). Waller-Duncan's multiple-range test was used to compare differences among cultivars or species means. Mean ranking sequence of root damage and leaf retention for each species or cultivar were plotted against nematode reproduction, log(Rf+1). Values for the uninoculated plants of each species or cultivar were also compared for growth vigor assessment. Correlation analysis among each of the relative parameters (relative weight gain ratio, relative root damage, relative gain in root number, relative symptom index, relative leaf retention, and nematode reproductive factor) and non-relative parameters (weight gain ratio, root damage, gain in root number, symptom index, and leaf retention) of the uninoculated were conducted.

6.4. Results

Screening Method

CBN infection decreased plant weight, root number, and leaf retention in most of the *Anthurium* species tested except *A.ravenii* and 'Mauna Kea' (Figs. 6.1.A, B). Adjustment of the parameters to the initial (before nematode inoculation) and the uninoculated treatment measurement enabled differentiation among the genotypes for tolerance to nematode infection ($P<0.05$). These parameters included relative gain in root number, root damage and leaf retention (Figs. 6.1.A, B, C). The higher the relative value, the higher the difference between inoculated and uninoculated plants. This indicated a lower

tolerance. A negative value of the relative parameters indicated a higher value for the inoculated than the uninoculated plants.

CBN tolerance and resistance among species was further evaluated relative to the standard reference *Anthurium* cultivars. The intolerant reference standard, 'Ozaki', was uniformly ranked low in plant tolerance among the species tested, as shown by the root damage and leaf retention differences (Figs. 6.1.C, 6.2). The tolerant reference standard, 'Midori', was ranked second lowest in terms of ranking of relative root damage (Fig. 6.2.A) but was ranked moderately tolerant in terms of relative leaf retention (Fig. 6.2.B). A relatively unstable tolerant cultivar, 'Mauna Kea' as determined in Chapter 3, had the highest tolerance based on mean relative root number, root damage, and leaf retention.

Identification of Tolerant Species

The CBN tolerant species identified from this experiment included *A. pittieri, A. ravenii*, *A. antioquiense*, and *A. aripoense*. *A. pittieri* had the lowest ranking of relative root damage (Fig. 6.2.A) and relative leaf retention (Fig. 6.2.B), indicating that it was most tolerant to CBN among the species tested. *A. ravenii* ranked closed to 'Midori' in root damage (Figs. 6.1.A, 6.2.A) and lower than 'Midori' in leaf retention (Figs. 6.1.B, 6.2.B). Therefore, *A. ravenii* was also considered as CBN tolerant. *A. antioquiense* and *A. aripoense* had lower relative leaf retention (Figs. 6.1.B, 6.2.B) but higher ranked root damage than 'Midori' (Figs. 6.1.C, 6.2.A), and thus were not considered as tolerant as *A. pittieri* and *A. ravenii*.

CBN tolerance could be due to nematode resistance as nematode reproduction was positively correlated with relative symptom index (P=0.0009, correlation coefficient=0.5) and relative leaf retention (p=0.002, correlation coefficient=0.45).

CBN tolerance can also be related to plant vigor and root number. This is indicated by a positive correlation between the plant vigor index (illustrated by symptom index, the lower the symptom index the higher the plant vigor index) of the uninoculated plants and

the relative root damage after nematode inoculation (P=0.004, correlation coefficient=0.43). 'Midori' and *A. aripoense* had relatively higher plant vigor index (lower symptom index) than the other species or cultivars tested (Fig. 6.3.A). *A. ravenii* Croat and Baker, *A. pittieri* Engl., *A. aripoense* and 'Mauna Kea' were among the species that had relatively low gain in root numbers among the uninoculated plants (Fig. 6.3.B).

Identification of Resistant Species

Nematode reproduction was lower in this experiment [log (Rf+1)<1] (Fig. 6.2) as compared to our previous in vitro screening [5<log (Rf+1)<20] (Fig. 3.1). A nematode population crash was not observed in this experiment, as nematode reproduction was highest in 'Ozaki'. Thus, screening for nematode resistance was reliable. 'Ozaki' in this case can be treated as a relatively susceptible reference standard.

A. ravenii, A. aripoense, A. scherzerianum, A.bicollectivum, and *A. pittieri*, were less susceptible than 'Ozaki' (P<0.05) (Fig. 6.4) and had nematode reproduction [log(Rf+1)]<0.3. This value gave a final nematode population lower than the initial nematode inoculation density, as would be expected for resistant germplasm. The relative resistant species among those tested was not due to nematode repellent effects; a positive correlation existed between the nematode reproduction in the medium and those in the tissue (P=0.005, correlation coefficient=0.4).

6.5. Discussion

Screening Method

Evaluation parameters used in this experiment improved our previous CBN screening efficiency. Adjustment of tolerance measurements to initial and the uninoculated control measurements better differentiated the genotype tolerance to CBN infection. Transformation of the parameters into non-parametric analysis (ranking sequence) is not necessary as in our previous in vitro screening results (Chapter 3). However, non-

parametric analysis is preferred as it compares the species or cultivars tested within a replication.

The relative intolerance of 'Ozaki' confirmed the reliability of this screening system, though a nematode population different from that used in previous in vitro screening was employed. 'Mauna Kea' performance was uniform (no difference within replications, $p>0.05$). This could reflect the effects of the adjustment of the parameters or use of a different nematode population.

Screening for CBN resistance was previously suggested to be conducted during a shorter period to avoid a nematode population crash (Chapter 3). However, screening for resistance was possible in this trial as no nematode population crash was observed in the intolerant reference standard, 'Ozaki', 3 months after nematode inoculation. This was due to the lower nematode reproduction [$\log(Rf+1)<1$] in this trial as compared to $5 < \log(Rf+1) < 20$ in our previous screening.

This screening system confirmed population variations in anthurium burrowing nematode reproductive rate. Tolerance to CBN might have slight variation as shown in the case of 'Mauna Kea'. However, 'Ozaki' remained a relatively intolerant cultivar regardless of the nematode population screened. This nematode population with slower nematode reproduction enabled concurrent screening for tolerance and resistance.

Mechanisms of Tolerance

Trudgill (1991) summarized that nematode tolerance is related to (1) nutrient, root growth, top growth, assimilation, respiration, dry matter partitioning or other growth and activity characteristics; (2) nematode parasitic effects; and (3) impaired root function and physiological effects. In addition, tolerance of potato genotypes to potato cyst nematode, *Globodera pallida,* was reported to be associated with the rate at which they induced hatching and the growth of roots at inoculation (Arntzen et al., 1994). Tolerance of the *Anthurium* species screened in this experiment was due to strong plant vigor, fewer target

roots for nematode infection, or lower nematode reproduction (higher nematode resistance).

Among the tolerant species or cultivars tested, *A. pittieri, A. ravenii,* 'Midori' and 'Mauna Kea' were relatively more vigorous, as determined by their relatively higher weight gain, and higher plant vigor index (lower symptom index) of the uninoculated plants. *A. ravenii, A. aripoense,* and 'Mauna Kea' gained fewer roots than standards but still remained tolerant. This is consistent with Trudgill's work on grafting experiments between potato cultivars, 'Pentland Dell' and 'Cara' (Trudgill, 1987). He showed that the scion contributed most to overall growth and partitioning, though both scion and stock contributed to tolerance of potato cyst nematode. Another explanation for the high tolerance in the species with fewer roots is that they provide fewer targets for nematode infection. This is confirmed by the lower nematode reproduction rate in *A. ravenii, A. aripoense,* and 'Mauna Kea'.

Identification of Resistance

Screening for resistance is possible by this method. Some of the species or cultivars had nematode reproduction of $\log(Rf+1) < 0.3$, which will give a $Rf < 1$. This demonstrated a reduction in nematode density from the initial inoculated nematode population. However, since the nematode infection rate is not known (i.e., the 400 nematodes used initially might not have all infected the plant tissue), the value of $Rf < 1$ might not represent resistance. Although *A. ravenii, A. aripoense, A. scherzerianum, A. bicollectivum* , and *A. pittieri* were among the species with lower nematode reproduction, *A. scherzerianum* and *A. bicollectivum* were not considered to be relatively resistant species due to their higher relative root damage or lower leaf retention than 'Ozaki', respectively. *A. ravenii* had the lowest nematode reproduction rate among the species tested followed by *A. aripoense* and *A. pittieri.* Thus, *A. ravenii* would be a potential candidate for CBN resistance breeding.

In conclusion, the nematode population with slower nematode reproduction in this experiment enabled concurrent screening for tolerance and resistance to *R. citrophilus* in *Anthurium*. This adds to the advantages of screening for CBN resistance in *Anthurium* species, as no relatively resistant cultivars were previously identified (Chapter 3). From this screening, *A. pittieri* and *A. ravenii*, were more tolerant than the reference standard, 'Midori'. *A. ravenii* was among the most resistant species followed by *A. aripoense* and *A. pittieri*. *A. ravenii* is already listed as an *Anthurium* species of interest in *Anthurium* breeding program (Kamemoto and Kuehnle, 1996). The CBN resistance and tolerance of *A. ravenii* will add to its favorable horticultural characteristics. Incorporation of these characteristics to *A. andraeanum* is likely to be possible as they belong to the same section, i.e. hybridization is possible. CBN resistance in *A. aripoense* might also be transmitted into *A. andraeanum* since hybrids between section *Calomystrium* and section *Belolonchium* had been reported (Kamemoto and Kuehnle, 1996). Though no hybrid between *A. pittieri* (section *Oxycarpium*) and *A. andraeanum* had been documented (Croat and Sheffer, 1983), crossability might be possible as they have the same chromosome number (Table 6.1). If these species are not crossable, embryo rescue (Matsumoto, 1994) might be an alternative way to incorporate the resistance and tolerance into *A. andraeanum*.

Table 6.1. Characteristics of *Anthurium* species screened in vitro for resistance and tolerance to *Radopholus citrophilus*.

Section and species	Univ. of Hawaii Accession number	Chromosome number (2n)	Root system	Distribution[a]	Spathe/Spadix/Fruit[a]
Porphyrochitonium					
A. antioquiense Engl.	A534	30 (Sheffer and Croat, 1983)		Northern Colombia	white spathe, lavender spadix
A. bicollectivum Croat	seed of A231	28, 29+1B+, 30 (Sheffer and Kamemoto, 1976)	moderately few, thin, green	Panama	spathe moderately thick, pale green; spadix yellow-green, yellow-orange berries
A. scherzerianum Schott	A614	ca. 30 (Gaiser, 1927)	numerous, thin	Costa Rica	spathe moderately thick, large showy, orange or red; spadix pale orange to red, soil; berries orange to red
Calomystrium					
A. ravenii Croat & Baker	A224	30 (Marutani, 1993)	relatively few, green to greenish-brown	Panama	spathe greenish white, tinged purplish at base; spadix cream, white; bright red berries
A. andraeanum Lind.ex André		30 (Marutani, 1993), 30+2 B (Kaneko and Kamemoto, 1978)	-	Ecuador and Colombia	wide range of spathe and spadix color; yellow berries
Belolonchium					
A. aripoense N.E. Br.	A193	6X=90			
Oxycarpium					
A. pittieri var. *pittieri* Engl. var. *morii* Croat	A269	30 (Sheffer and Kamemoto, 1976)	slender, relatively numerous at the lower nodes, few or none from upper nodes	Panama or Costa Rica	spathe green, moderately thin; spadix slender, gradually tapered toward apex, green yellow berries in Panama, or white in Costa Rica

[a] Croat, 1983; Croat, 1986; Croat and Sheffer, 1983; Kamemoto and Kuehnle, 1996.

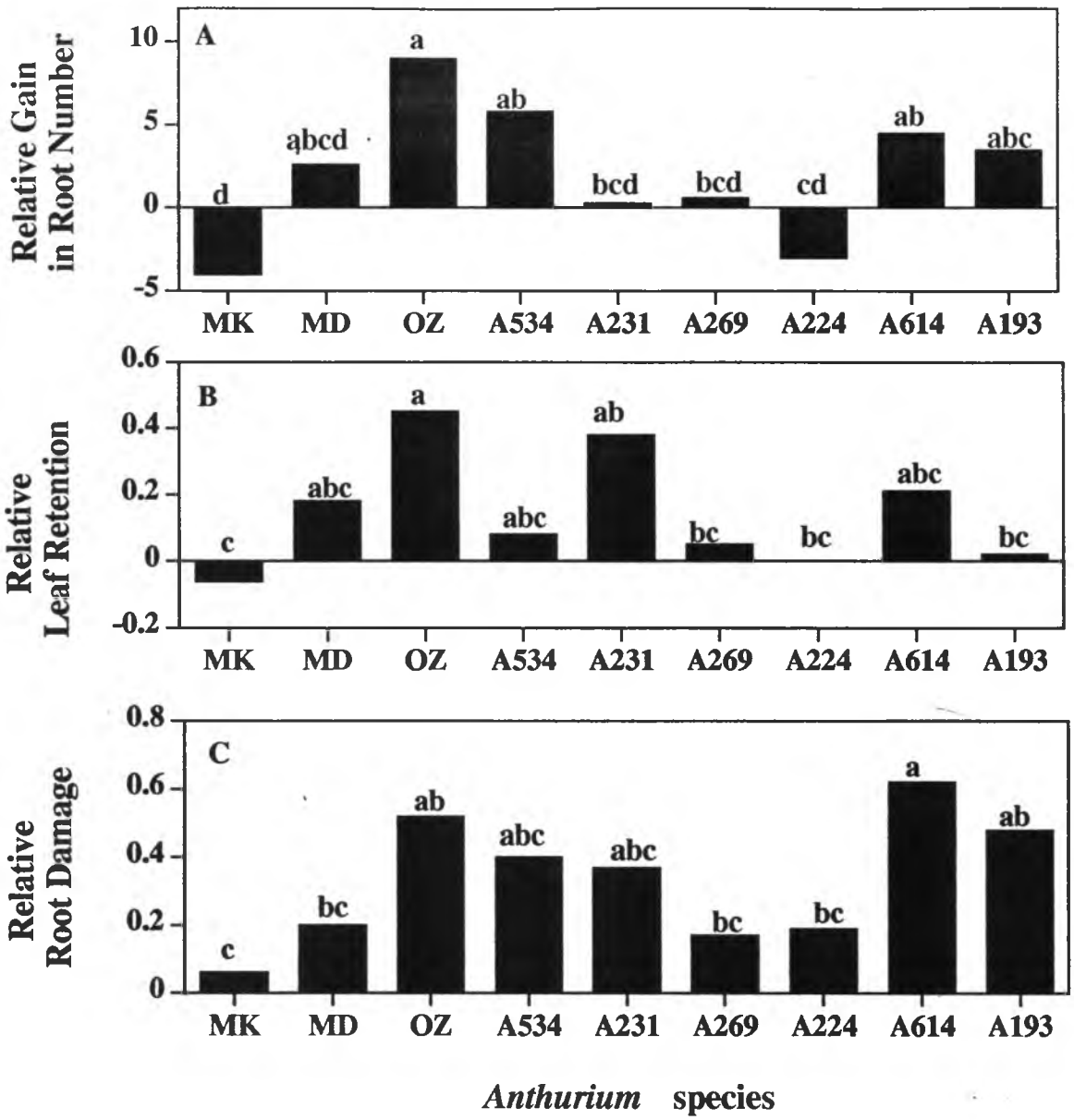

Fig. 6.1. Means of (A) relative gain in root number (difference between initial and final root number), (B) relative leaf retention (percentage of green leaf divided by total number of leaf) , and (C) relative root damage (number of black root divided by total root number) of 'Mauna Kea' (MK), 'Midori' (MD), 'Ozaki' (OZ), *A. antioquiense* (A534), *A.bicollectivum* (A231), *A. pittieri* (A269), *A. ravenii* (A224), *A. sherzerianum* (A614) and *A. aripoense* (A193) 3 months after *Radopholus citrophilus* inoculation. Relative refers to the difference between inoculated and uninoculated plants. Columns with the same letters are not significantly different (P=0.05). Values are averages of 6 replications.

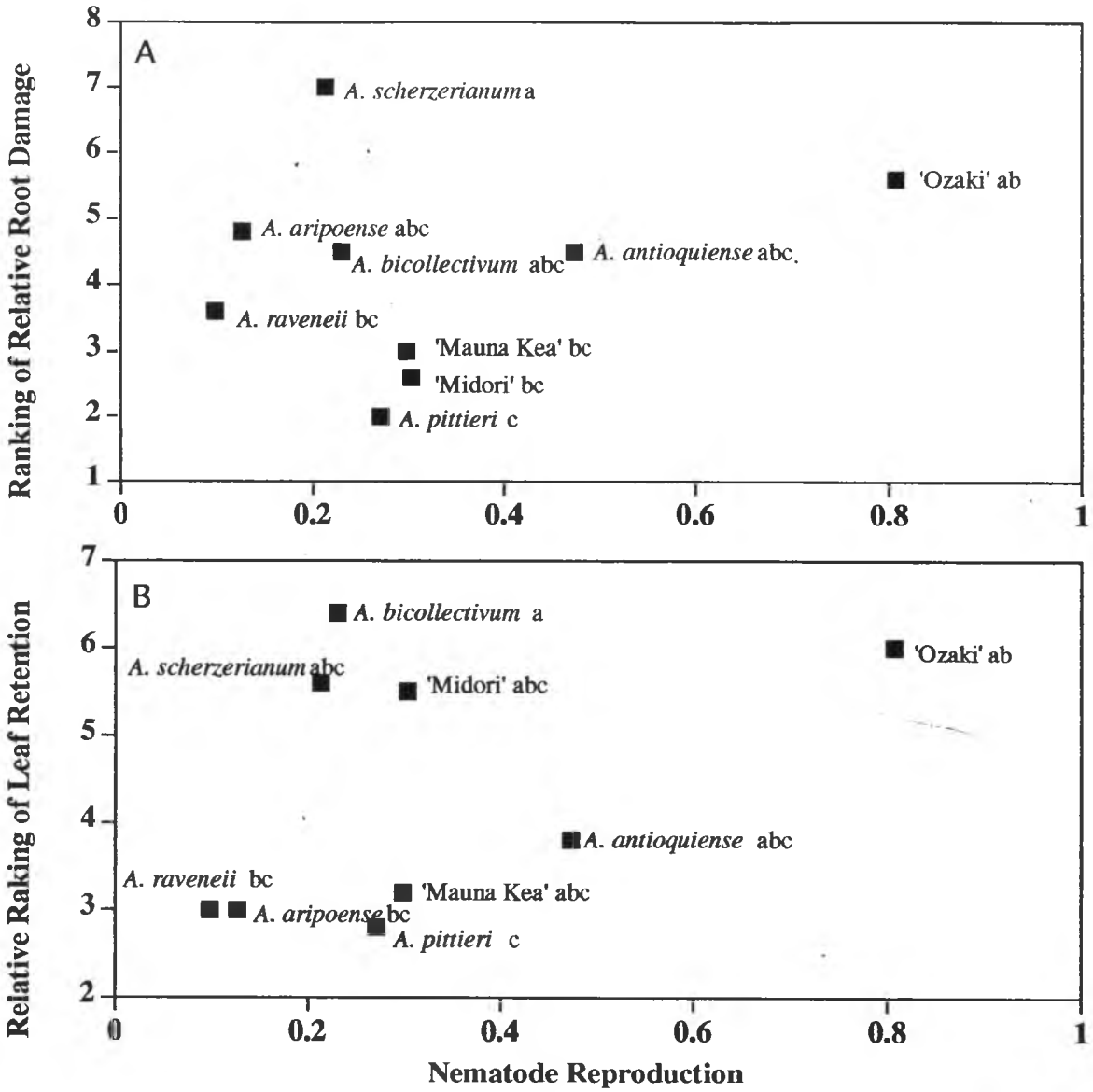

Fig. 6.2. Plot of (A) ranking of relative root damage (difference of root damage of the inoculated from the uninoculated, root damage=number of black root divided by total number of root), (B), ranking of relative leaf retention (difference of leaf retention of the uninoculated from the inoculated) against nematode reproduction, log(Rf+1) (Rf=Final nematode number divided by initial nematode number), of 9 *Anthurium* species or cultivars 3 months after *Radopholus citrophilus* inoculation in vitro. Data points with the same symbol are closely related in terms of their tolerance measurement and nematode reproductive factor. Data points followed by the same letter are not different from each other in terms of the tolerance measurement ($P<0.05$), and are means of the ranking of 6 replications.

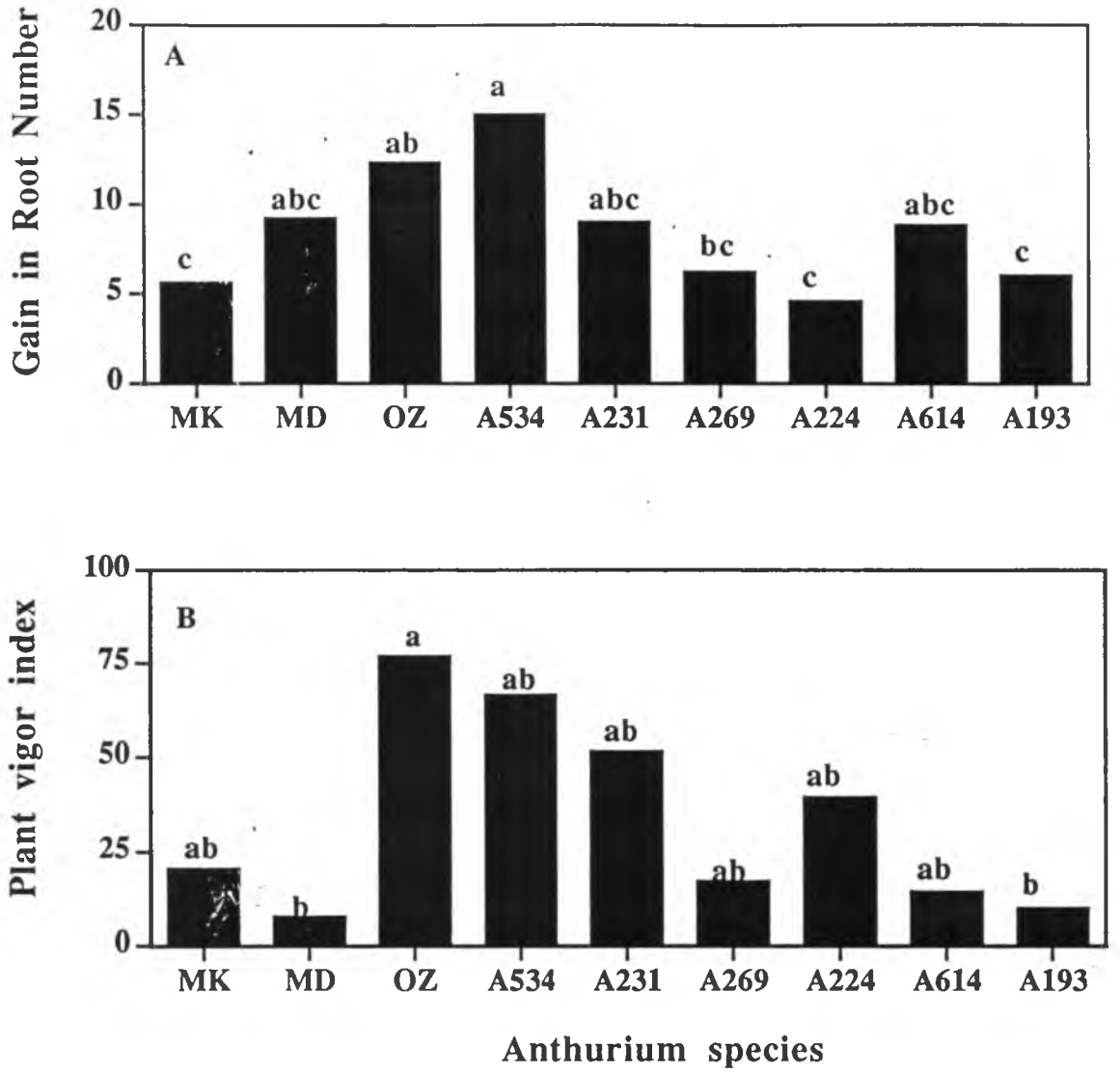

Fig. 6.3. Performance of *Anthurium* plants, in vitro, uninoculated with *Radopholus citrophilus* in terms of (A) gain in root number (B) plant vigor index (summation of percentage of damage leaf × leaf damage level) in 'Mauna Kea' (MK), 'Midori' (MD), 'Ozaki' (OZ), *A. antioquiense* (A534), *A.bicollectivum* (A231), *A. pittieri* (A269), *A. ravenii* (A224), *A. scherzerianum* (A. scher.) and *A. aripoense* (A193) after 3 months. Columns with the same letters are not significantly different (P=0.05). Values are average of 6 replications.

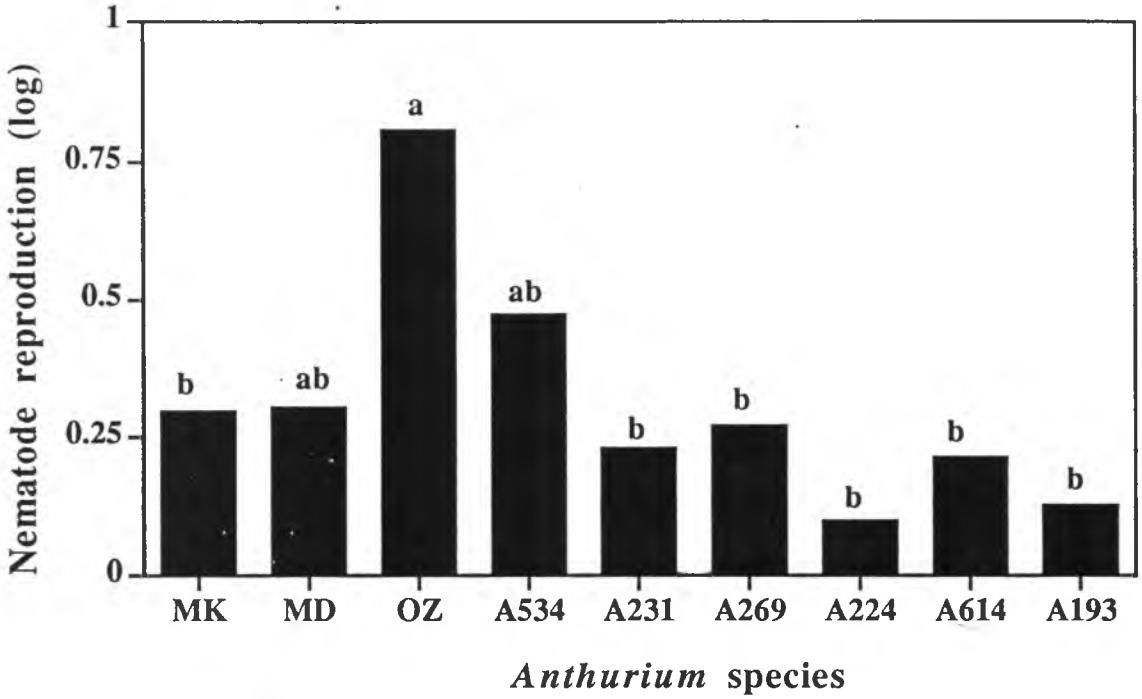

Fig. 6.4. Nematode reproduction, log(Rf+1), where Rf is final nematode number divided by initial nematode number, 3 months after *Radopholus citrophilus* inoculation in 'Mauna Kea' (MK), 'Midori' (MD), 'Ozaki' (OZ), *A. antioquiense* (A534), *A. bicollectivum* (A231), *A. pittieri* (A269), *A. ravenii* (A224), *A. scherzerianum* (A. scher.) and *A. aripoense* (A193). Columns with the same letters are not significantly different (P=0.05). Values are averages of 6 replications.

6.6. Literature Cited

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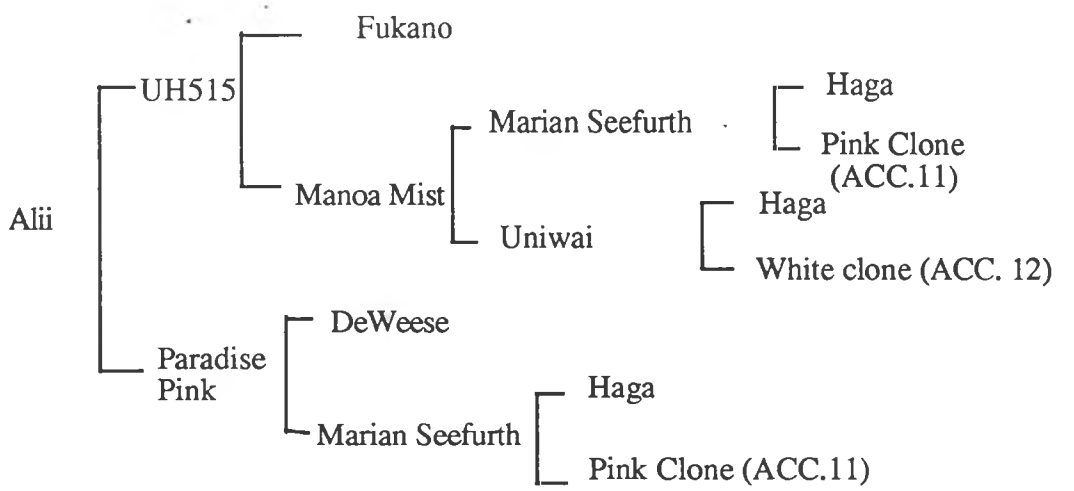
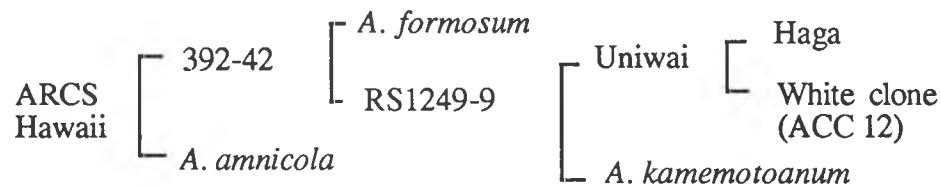
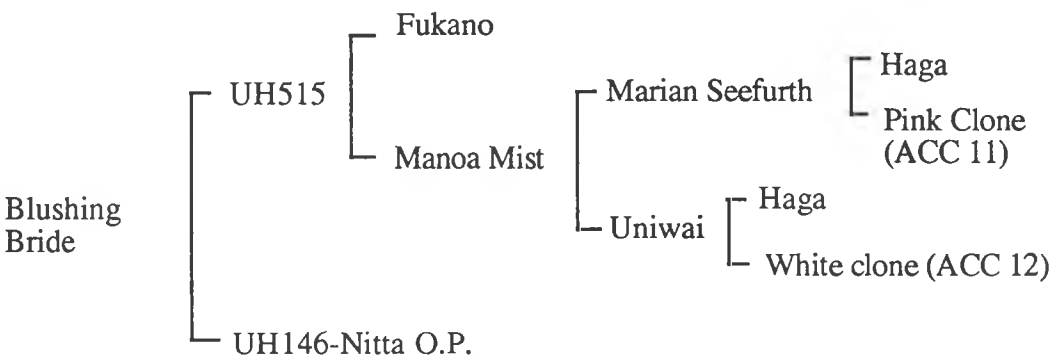
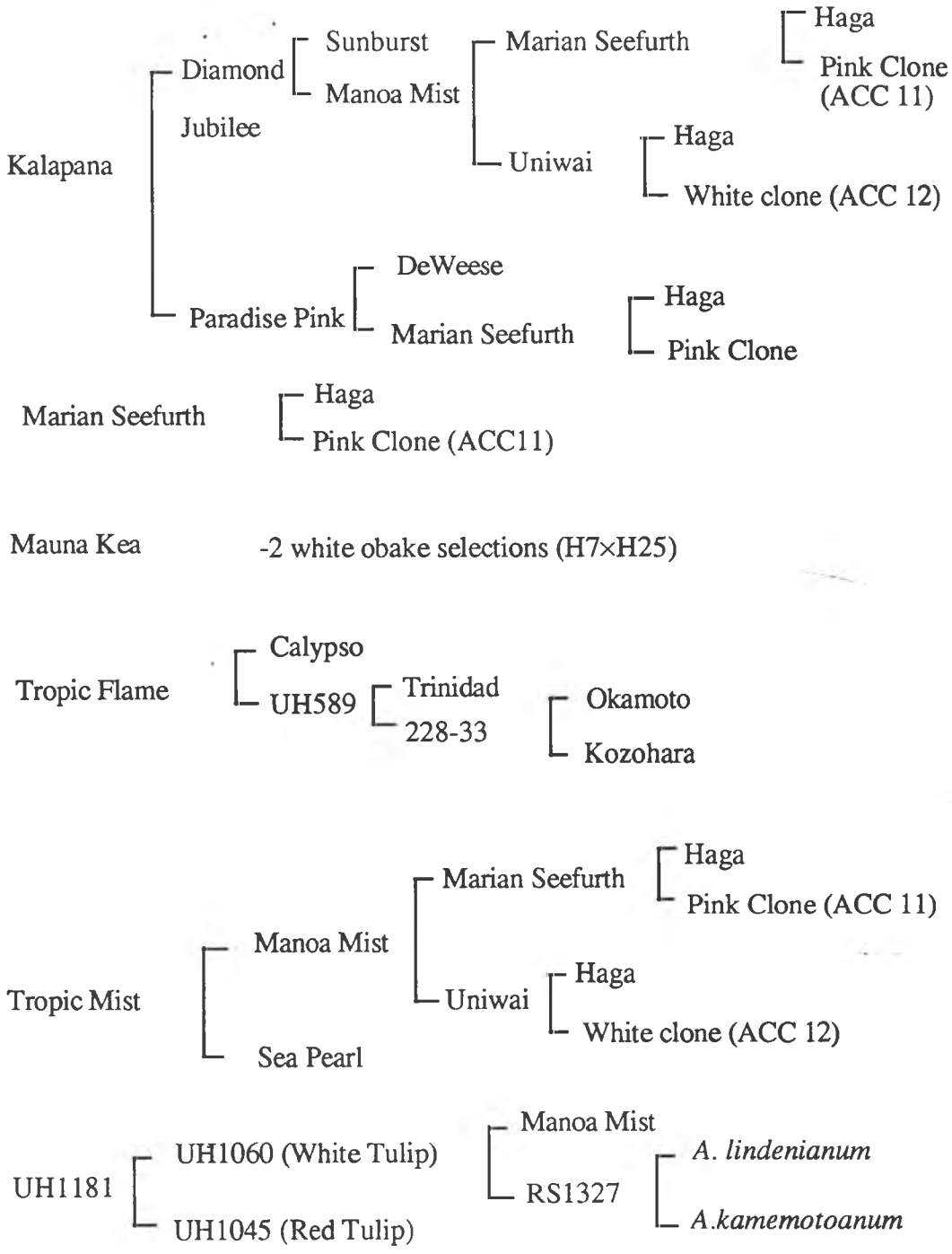
Appendix A. Genetic Background of *Anthurium* Cultivars








Kalapana
Diamond
Sunburst
Manoa Mist
Jubilee
Marian Seefurth
Haga
Pink Clone (ACC 11)
Uniwai
Haga
White clone (ACC 12)
Paradise Pink
DeWeese
Marian Seefurth
Haga
Pink Clone
Marian Seefurth
Haga
Pink Clone (ACC11)
Mauna Kea
-2 white obake selections (H7×H25)
Tropic Flame
Calypso
UH589
Trinidad
228-33
Okamoto
Kozohara
Tropic Mist
Manoa Mist
Sea Pearl
Marian Seefurth
Haga
Pink Clone (ACC 11)
Uniwai
Haga
White clone (ACC 12)
UH1181
UH1060 (White Tulip)
UH1045 (Red Tulip)
Manoa Mist
RS1327
A. lindenianum
A.kamemotoanum

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