ANTHURIUM FRAGRANCE: GENETIC AND BIOCHEMICAL STUDIES

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ABSTRACT

The total of 147 *Anthurium* species and hybrids at the University of Hawaii and the Missouri Botanical Garden germplasm collections was evaluated for the presence or absence of scent, type of fragrance, time of emission, daily occurrence, developmental stage of scent emission, color of spathe and spadix. A majority of inflorescences (76%) emitted scent. Scent was categorized as citrus, fishy, floral, foul, fruity, menthol, minty, pine, spicy and sweet. There was no relationship between scent production or scent quality with flower color or botanical section. A plurality of inflorescences emitted scent during the morning (45%) and at the pistillate stage (77%). Fragrance life of unharvested inflorescences varied from 2 to 3 days up to 4 weeks, whereas that of harvested inflorescences was only 1 or 2 days.

F_1 progenies of crosses between fragrant × fragrant and non-fragrant × fragrant parents were studied to determine whether a single gene or more than one gene governed presence of scent. Progenies from 24 crosses were tested by Chi-square analysis for a single dominant or recessive trait. None produced expected segregation ratios for a single gene trait, indicating that multiple genes likely govern presence of fragrance in *Anthurium*. Progeny also segregated for fragrance quality and included non-parental scents.

Fragrance of seven *Anthurium* species and ten hybrids was analyzed by gas chromatography and mass spectrometry. Nineteen monoterpenes (lipids) and some alcohols, aldehydes and esters were identified. Limonene and 1,8-cineole were common

to most samples along with α-pinene, β-pinene, myrcene and linalool. Hybrid UH1299, emitting a sweet and floral scent all day, showed fluctuation in amounts and types of compound during the daily cycle.

Tepals were associated with fragrance production in *Anthurium*. Histological comparison between tepals of fragrant and non-fragrant spadices showed lipids and starches present in both fragrant and non-fragrant samples. However, in fragrant samples, the amount of lipids was significantly greater than that of non-fragrant ones, whereas the amount of starch was significantly greater in non-fragrant samples compared with fragrant ones. These data support the hypothesis that high levels of lipids were associated with fragrance production.

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LITERATURE REVIEW

1.1 Sense of Smell

1.1.1 How Do Human Beings Perceive Odor?

Odor is basically the result of an interaction between a chemical stimulus and an olfactory receptor system causing biological and psychological effects in a living organism. Almost any type of chemical molecules can act as odor stimuli if those molecules are sufficiently volatile to be present in the air (Neuner-Jehle and Etzweiler, 1991). In daily life, humans breathe about 17,280 times and move around 438 cubic feet of air. It takes about 2 seconds to inhale and 3 seconds to exhale; odor molecules pass through the olfactory system during inhalation (Ackerman, 1991). The odor molecules move to the olfactory epithelium with receptor cilia in the upper part of the nose. Contact between the odor molecules and the receptor cells creates a signal which is transferred to the limbic system, that part of the brain involved with emotions and memories of sense (Neuner-Jehle and Etzweiler, 1991). After this system has been stimulated, it can activate the hypothalamus and pituitary gland to stimulate production of hormones controlling sex, appetite, body temperature and other functions. The limbic system also reaches into the neocortex, part of the brain involved in discrimination of fine odor, to stimulate conscious thoughts and reactions (Gibbsons, 1986).

1.1.2 Terminology

Several terms refer to odors from different sources. Generally, odor defines things that stimulate the olfactory organ (Meilgaard *et al.*, 1991). Aroma is the odor of food products whereas fragrance is the odor of a perfume or cosmetic (Meilgaard *et al.*, 1991). Fragrance can also apply to sweet delicate odors such as from fresh flowers or pine trees. Scent is the characteristic smell given off by a substance, animal or plant (Merriam-Webster, 1991). Aromatics are the volatiles that the olfactory system perceives from compounds in the mouth (Meilgaard *et al.*, 1991). The term "essential oils" is used to refer to any class of volatile oils from plants (Merriam-Webster, 1991). The terms scent and fragrance will be used interchangeably in this dissertation to refer to the odor of *Anthurium*.

1.1.3 Factors Affecting Sense of Smell

The amount of volatile compounds and their volatility affect perception of scent by the human nose. The first factor can be affected by temperature and the chemical structure of the compounds (Meilgaard *et al.*, 1991) whereas volatility is influenced by a surface condition. At a given temperature, more volatiles escape from a soft, porous and humid surface than from a hard, smooth and dry one.

The olfactory sensibility of humans is also subject to change over time. This might relate to hormonal influences as well as to aging, especially after age 60 (Calkin and Jellinek, 1994). The sensitivity of nose receptors to different chemicals varies over a range of 10^{12} molecules or more. Sensitivity of the human nose to various odors may be

measured by dual flow olfactometry using *n*-butanol as a standard. Subjects show varying sensitivity to odors depending on hunger, satiety, mood, concentration, presence or absence of respiratory infections and, in women, menstrual cycle and pregnancy (Meilgaard *et al.*, 1991).

Moreover, psychological factors and individual experience can influence interpretation of smell (Civille and Close, 1994). Since childhood, the sense of smell developed parallel to the development of the brain. The more a person is exposed to a variety of odors, the more she/he learns about odors.

1.1.4 Flower Scent

Several thousand compounds have been identified from various floral scents. Analysis is mostly done by steam distillation or headspace entrapment in combination with gas chromatography and mass spectrometery (GC-MS) (Knudsen *et al.*, 1993). Most floral scent compounds are terpenoids, benzenoids or fatty acid derivatives (Croteau and Karp, 1991; Knudsen *et al.*, 1993). Included among the terpenoids are limonene, myrcene, linalool, pinenes, ocimenes and 1,8-cineole. Included among the benzenoids are methyl-2-hydroxybenzoate, benzaldehyde, benzyl alcohol, benzyl acetate, 2-phenylethanol and methyl benzoate. The fatty acid derivatives commonly comprise both saturated and unsaturated hydrocarbons as well as aldehydes, alcohols and ketones.

Fragrance terpenoids can be divided into four major groups: irregular terpenes, monoterpenes, sesquiterpenes and diterpenes (Fig. 1.1). Common irregular terpenes are ionones, 6-methyl-5-hepten-2-one, and geranyl acetone. Monoterpenes comprise two

types, monoterpene hydrocarbons and oxygenated monoterpenes. The odor of monoterpene hydrocarbons is spicy or resinous whereas that of scent oxygenated monoterpenes is sweet or citrus-like. Sesquiterpenes are also found in scented flowers and vegetative parts, with the two most common sesquiterpenes being caryophyllene and α-farnesene. Very few diterpenes have been reported in floral scents, presumably due to the large amount of carbon which decreases the volatility of these compounds (Knudsen *et al.*, 1993).

1.1.5 Importance of Flower Scent in Plant Biology

Ecologically, fragrance emission is significant for attraction of pollinators (Meeuse and Morris, 1984). Flowers may produce chemicals as "scent guides" on the flower surface to aid the pollinator to locate and recognize a particular flower (Robacker *et al.*, 1988). Specific scents may also enhance the fidelity of insects to particular flower species as well as to increase the efficiency of intraspecific pollen transfer. Robacker *et al.* (1988) reported that floral scent may serve as a dual olfactory communication system. By emitting specific scent components, flowers signal pollinators when maximum nectar is available. On the other hand, flowers release scent at the receptive stage even though visiting pollinators come to visit flowers without any nectar reward. In addition, some flowers produce scent that mimic pheromones to attract pollinators (Dafni, 1992).

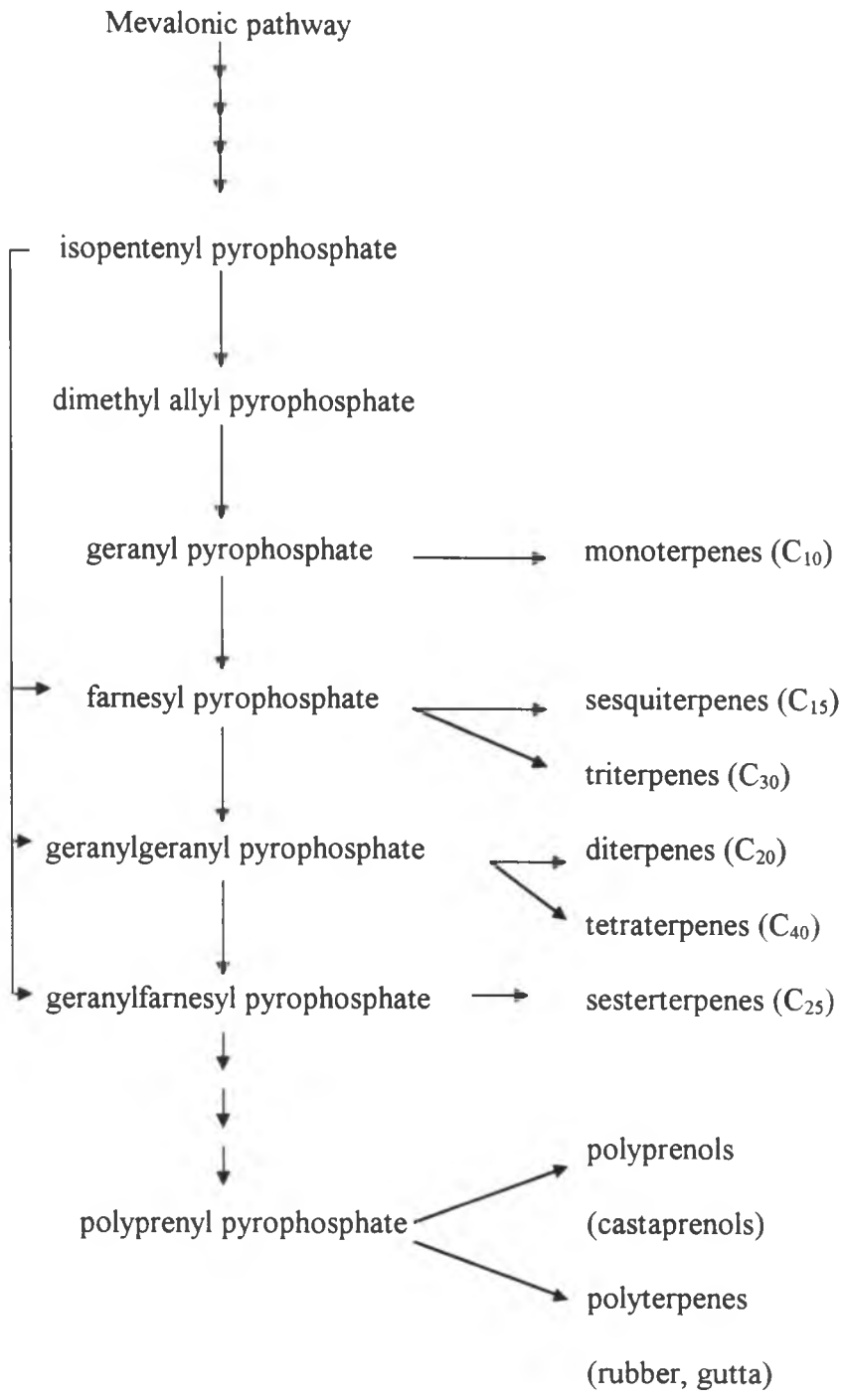

Figure 1.1 Illustrated terpene biosynthesis pathway, from mevalonic acid on (from Gershenzon and Croteau, 1990).

1.2 Fragrance Classification

Classification of fragrance varies with the type of the material or the application of fragrance. For example, rose scent had been classified into eight types: wild rose or species, spicy, tea, flower, old rose, fruity, berry and briar (Forrest, 1945). Delbard developed a system to describe rose flower scent as: a) head fragrance, which is the first fragrance that evaporates, comprised of citrus and aromatic families; b) heart fragrance, comprised floral, fruity, herbal and spicy families; and c) base fragrance comprised of woody and balsamic families (Jasik, 1993).

Classification of scent for use in perfumery is more specific. Roudnistka (1991) described raw material used in perfumery with 15 categories, namely citrus, rose, orange, jasmine, violet, aniseed, aromatic, green, spicy, woody, tobacco, fruity, balsamic, animal and leathery. Calkin and Jellinek (1994) classified raw materials used in perfumery into 30 groups, namely aldehyde, amber, anisic, aromatic-herbal, balsamic, camphor-cineol, cinnamic, citrus, floral, floral fresh, floral balsamic, fresh, fruity, green, herbal, iris, lavender, leather, linalool, minty, mossy, musk, patchouli, pine, resins, seeds, spicy, sweet, watermelon-cucumber and woody. The odors of commercial products such as car air freshener or soap were categorized by Civille and Close (1994) as aldehydic, camphoraceous, citrus, floral, green, herbaceous, leather/animal, mint, moss/chypre, ozonic/marine, pine, resin, spice, sweet and wood.

An individual's description of an odor is based on olfactory memory, knowledge of raw material and experience (Thiboud, 1991). Panel training can then be conducted

towards certain applications. Although it is not reported, such panel training could also be directed towards descriptive analysis of flower fragrance (Meilgaard *et al.*, 1991). The assembly of a sensory evaluation panel requires candidates with good odor perception and a qualified leader for conduction of the panel training. Test samples and possibly standards should be consistent and abundant to allow ready access by each panelist during the training and test periods.

Two common experimental methods used in sensory evaluation are measured responses and difference tests (Civille and Close, 1994). In measuring response, a panel will evaluate test samples and rate individual samples based on a scale, for example, from 0 to 9 or from none to strong, depending on the measurement. Data can be analyzed using χ^2-tests, t-test or analysis of variance depending on the categories among a group of samples. In difference tests, the objective is to determine a difference among test samples. A panel will choose samples that are different among the test samples or test sample that is similar to the references. Data are evaluated using analysis of variance (ANOVA).

For descriptive analysis, panelists have to develop descriptors by evaluating a broad array of tested samples, then develop proper descriptions for particular samples in order to understand and evaluate other subsequent samples accordingly. To develop flower fragrance description, for example, panelists need to smell all types of fragrance and then list all possible terms used by the panel. Then, panelists discuss and decide on the description of each category. In this manner, panelists develop a shared understanding of an odor (Meilgaard *et al.*, 1991; Civille and Close, 1994).

1.3 Fragrance Analysis

1.3.1 The Chemistry of Volatile Compounds

Volatile compounds are the chemical molecules that vaporize at a relatively low temperature. In general, volatility can be determined by the number of carbon atoms present in a compound. The higher the number, the lower the volatility of the compound. However, the presence of a functional group on a molecule can reduce the volatility. This effect is due to polarization of the electrical charges within the molecules. For an equivalent molecular size, hydrocarbons and ethers are more volatile than aldehydes, ketones and esters, which in turn are more volatile than alcohols and acids. In perfumery, the volatility of a substance can be determined by measuring its vapor pressure at room temperature (Calkin and Jellinek, 1994).

1.3.2 Extraction

1.3.2.1 Solvent Extraction

In solvent extraction, raw materials are soaked in a particular solvent for a certain period of time, depending on the type of tissue being analyzed. Flowers or leaves require shorter soaking times than bark. The solvent must be miscible with essential oils and have a low boiling point. This method usually applies to raw material that is very sensitive to heat (Morris, 1984). Common solvents employed in extraction are dichloromethane, ether, hexane, methylene chloride and pentane (Dobson, 1991).

1.3.2.2 Enfleurage

Fats have long been used to capture odors of nature, especially flower fragrance. In enfleurage, usually lard and tallow are mixed in a two to one ratio and then poured into a wooden frame and set aside until solidified. Fresh flowers, perfectly dry, are left on top of the fats for at least 24 to 48 hours. After that, flowers are removed, the fats are gently warmed to melting and then transferred through gauze to remove all the flower residue and left to cool. A solvent such as ethyl alcohol is used to extract essential oils from the fat mixture. After the solvent is added and mixed well, the mixture is chilled, fats are solidified and then removed (Morris, 1984).

1.3.3 Distillation

The principle of this method is very simple. Raw materials are placed in a still, either in water or on a screen above water, for steam distillation. At the top of the still is a duct that connects to a condenser pipe with a cooling system, to condense any vapors produced from the boiling pot. The vapors contain both essential oils, gases and water vapor. As they move along the cooling system, they condense and drop to the container below, with water and essential oils separating due to differences in density. While most essential oils float on top of water, some such as clove and anise drop to the bottom. This method might not be appropriate for certain types of raw materials that are very sensitive to heat, for example jasmine and narcissus (Morris, 1984).

1.3.4 Headspace

To develop a new generation of perfumes more closely related to natural odor, as well as to search for new fragrance substances and new ideas for creating perfume components, natural flower fragrance needs to be analyzed (Surburgh *et al.*, 1993). Classical methods such as extraction and distillation yielded products did not reproduce the sensory properties of the natural material. To isolate the volatile constituents for analytical and perfumery purposes, a new method called headspace was introduced to trap fragrant volatiles of flowers (Surburgh *et al.*, 1993).

An advantage of the headspace technique is that it is non-destructive. It can be used on living tissue either in the laboratory or in the field and the sampling can then be repeated over time on the same individual (Knudsen *et al.*, 1993). There are two types of headspace methods. In dynamic headspace, volatile compounds released by plants are purged by an airstream and trapped by adsorption. The adsorptive materials used may be charcoal, Tenax or similar macroporous resins. Then, in the second step, volatile compounds are obtained by desorption with solvent or by heating.

A second method, vacuum headspace is a form of vacuum steam distillation. Flowers are subjected to a partial vacuum during which volatile compounds and water distill off and are condensed at low temperature. By this method, volatile compounds are concentrated immediately (Surburgh *et al.*, 1993).

1.3.5 Chemical analysis

After volatile compounds are separated from flowers, they are injected into a gas chromatography (GC) or gas chromatography and mass spectrometry (GC-MS). GC analysis allows preliminary identification of selected constituents. Chemical identities of compounds are established using GC coupled with MS. Conditions used for the analysis by GC and GC-MS depend greatly upon the instrument, column characteristics and chemistry of the particular sample.

GC is a technique used to separate compounds based on their affinity to the stationary phase of a column (Fowlis, 1995). The greater the affinity of the compound for the stationary phase, the more the compound will be retained by the column. Compounds move in the column with the aid of a carrier gas such as helium or hydrogen, and based on temperature. At the end of the column is a detector, commonly a flame ionization detector, FID. When a compound elutes from the column, it is mixed with hydrogen, burned in the flame and then passed into the detector. Ions are generated and produced an ionization current which is fed to the data system.

MS is a technique used in determining molecular weight of compounds (Geddes, 1980). A MS machine is usually connected with a GC machine. After the gas molecule elutes out of the column of GC, it is bombarded with high energy electrons, accelerated from a hot filament. Some molecules lose electrons, promoting fragmentation. Generally, the electron energy used in MS analysis is 70 eV. The fragments move at high speed through electromagnetic lenses. The smaller the mass of

the fragment, the faster it moves to the detector. After reading the fragmented mass, the detector produces an electrical signal for analysis by a database system.

1.4 Scent Production in Flowering Plants

Production and accumulation of volatile compounds can be found throughout the plant kingdom. Flower scent is generally composed of a blend of several to many compounds, and each organ may have its own specific blend. In *Pyrola* spp., the major compounds found in the petals were phenyl propanoids whereas those found in the stamen were methoxy benzenes. In *Moneses*, both isoprenoids and benezenoids were found in stamens and petals but the proportion of benzenoids was greater in the former (Knudsen and Tollsten, 1991).

Plants produce essential oils in different type of structures. Most of them come from leaves and stems, such as from sandalwood, eucalyptus and peppermint. Only a few are obtained from flowers, such as from lavender, jasmine and rose (Hay and Svoboda, 1993). Most information about the tissue of scent production in flowering plants is limited to non-floral plant parts and few scent structures are recognized (Table 1.1). Osmophore is a term used to define a cell that acts as a secretory cell for scent emission or fragrance glands (Vogel, 1990). Floral structures involved in odor production vary from species to species (Dafni, 1992).

Terpene, for example, is a common volatile found in all plants (Harborne and Turner, 1984). Among the most important terpenes are the plant growth hormone

gibberellins (GA), diterpenes (C_{20}) occurring in more than 84 forms (Taiz and Zeiger, 1991). In gymnosperms, monoterpenes such as limonene, linalool and myrcene are commonly found. Quantitative variation of volatiles are used as taxonomic markers in species classification (Harborne and Turner, 1984). In angiosperms, both dicotyledons and monocotyledons accumulate terpenes.

Table 1.1 Some plant families producing scent compounds.

Family	Scented representative	Source of volatile compounds	Affiliated tissue or structure
Dicotyledons			
Apiaceae	coriander	leaf, seed	schizogenous ducts
Apocynaceae	plumeria	flower	-
Chenopodiaceae	beet, goosefoot	flower, leaf	-
Compositae	pyrethum	leaf	schizogenous ducts
Geraniaceae	geranium	leaf	-
Guttifurae/Hypericaceae	St John's wort	flower	-
Labiatae	mint	leaf, stem, flower	trichomes
Lauraceae	bay	leaf	-
Myriaceae, Myristicaceae	nutmeg, mace	fruit kernel	-
Myrtaceae	myrtle, eucalyptus, clove	leaf, flower bud	-
Oleaceae	jasmine	flower	-
Piperaceae	pepper	leaf, fruit	-
Rosaceae	rose	petal	papillae hair
Rubiaceae	gardenia	flower	-
Rutaceae	citrus	rind	-
	borania	flower	-
Santalaceae	sandalwood	bark	-
Verbenaceae	verbena	leaf, stem flower	-
Violaceae	violet, pansy	leaf, flower	-
Monocotyledons			
Araceae	skunk cabbage	spadix, spathe	osmophore
Cyperaceae	sedge	root	-
Gramineae	cymbopogon, grass, lemongrass	leaf	-
Iridaceae	iris	rhizome	-
Orchidaceae	orchid	sepal, lip (modified petal)	epithellium, osmophore
Zingiberaceae	ginger	rhizome	-

1.4.1 Apiaceae

The Apiaceae or Umbelliferae (carrot family) consists of about 300 genera and 3,000 species of herbs. Plants in this family produce volatile compounds such as monoterpenes, sesquiterpenes and phenylpropanoid compounds, and related resin in secretory ducts of roots, stems, leaves, inflorescences and fruits (Hay and Svoboda, 1993). Essential oils are synthesized in schizogeneous ducts that are lined with living mesophyll cells and organized into a secretory epithelium (Esau, 1977). The essential oils are released to the environment only when the plant tissues are damaged. Knowledge about schizogenous ducts is relatively scarce due to the complexity of internal structure, rendering them very difficult to study (Hay and Svoboda, 1993).

1.4.2 Araceae

There are about 105 genera and more than 3,000 species in this family (Boyce, 1995). Members possess the largest scent organ in the plant kingdom in the form of the spadix. Scent can be produced during different stages of reproductive development, for example, during the female receptive stage in *Spathiphyllum* or during anthesis in *Philodendron* (Vogel, 1990). Heat production also occurs during scent emission in some aroids such as in *Arum italicum*, *Arum maculatum*, *Dracunculus vulgaris Sauromatum guttatum* and *Symplocarpus foetidus* (Knutson, 1974; Smith and Meeuse, 1966).

In *Arum*, volatile compounds are accumulated in lipid vacuoles, such as spherosomes, in the epidermal protoplasm of the spadix. The volatile compounds pass from cell to cell through the plasmodesmata and are then released through the membrane

of papillate epithelium (Vogel, 1990). In *Arum maculatum*, heat production occurred in the male flower before the spathe unfolded (Bermadinger-Stabentheiner and Stabentheiner, 1995). During this period, a fruit-like scent was produced and pollen grains were released. The second stage of heat production occurred again when the spathe unfolded and at this time dung-like odor was produced. The papillate epidermal cells on the spadix and spathe were turgid before heat production, and they soon collapsed after heat production ceased.

In *Sauromatum guttatum*, division of mitochrondria and the presense of lipid bodies and peroxisomes were found in the epidermis of the spadix during heat and odor production from the osmophore (Skubatz *et al.*, 1993). Starch was stored for heat production. In addition, lipid bodies found in this species were identified as sesquiterpenes (Skubatz *et al.*, 1995). These compounds were present before scent production, and disappeared after scent was released.

In some species, such as *Arisaema* and *Cryptocoryne*, the source of scent production has shifted from the spadix to the spathe (Vogel, 1990).

1.4.3 Asteraceae

The Asteraceae or Compositae is one of the largest plant families among dicots, containing over 1000 genera and 25,000 species. This family includes a number of important food and industrial crop plants, weeds and species of herbal and agrochemical importance. The secretory structures in this family are broadly similar to the ducts in

Apiaceae. In some species, e.g. *Artemisia dracunculus*, the schizogenic ducts occur in association with glandular trichomes (Hay and Svoboda, 1993).

1.4.4 Labiatae

There are about 200 genera and 5000 species of herbs and low shrubs in the Labiatae family. Most labiates accumulate terpenes and a range of other compounds primarily in epidermal glands of leaves, stems and reproductive structures. In leaves, volatile compounds are stored in glandular trichomes that vary in terms of morphology among species as well as the number of glands per unit area in the epidermis. There are two types of trichome, capitate and peltate. Capitate is a trichome that has a single head cell whereas peltate is a complex head, comprised of four to ten cells per head. A glandular trichome develops from a single epidermal cell. The trichome has a larger nucleus, smaller central vacuole and more electron-dense cytoplasm than other regular epidermal cells. Essential oils are synthesized and accumulated in trichome cells. In capitate-type trichomes, essential oils are lost to the surrounding environment through pores in the cuticle of head cells. Essential oils in peltate type trichomes are accumulated inside the extracellular space between head cells and cuticle. The transport of essential oils out of the cells across plasmalemma membranes and into the extracellular storage spaces is not yet well understood. However, the accumulation of essential oils can not be detected in the cytoplasm (Hay and Svoboda, 1993).

1.4.5 Orchidaceae

There are more than 600 genera (Baumgardt, 1982) and about 30,000 species of orchids (Northen, 1990). Volatile compounds are released from osmophores that vary in terms of morphology and in their location within the flower (Vogel, 1990). For example, osmophores are found in the sepal of *Restrepia antennifera*, in the labellum of *Catasetum macrocarpum*, and in the lateral sepal of *Masdevallia nidifica*. In the osmophore, starch is accumulated in the tissue below the epidermis. It is metabolized to volatile compounds that are immediately released through the cuticle (Metcalf, 1987).

1.4.6 Rosaceae

Among the roughly 120 genera belonging to this family are included some important commercial crops such as apple, peach and rose (Heywood, 1985). The most used for its valuable essential oils is the genus *Rosa*. Rose oil derived from petals is a major industry of western Asia countries. Rose scent is released from the petal through papillae hairs (Morris, 1984).

1.5 Inheritance of Fragrance

Two scientific studies are published on the genetic control of flower fragrance. In gladiolus, two fragrant species were intercrossed and their F_1 and F_2 generations were evaluated for the study of fragrance inheritance. These two species were *Gladiolus tristis*, with a lemon-like fragrance during the night, and *G. recurvus*, with a violet scent during

the day. However, conclusions about the inheritance of the fragrance character could not be made as the test ratios did not fit the chi-square value for qualitative traits. Quantitative inheritance is thus a possibility (McLean, 1933).

Inheritance of flower fragrance in rice was also studied. The introduced rice cultivar, C. I. 3794, with a violet-like fragrant flower, was crossed with a non-fragrant flower cultivar. F_2 progenies were scored for the presence or absence of fragrance. It was found that the violet-like fragrance flower in this particular rice cultivar was controlled by a single dominant gene (Jodon, 1944).

Even though only two crops were studied for inheritance of flower fragrance, six crops were studied for the inheritance of plant odor.

1.5.1 *Chamomilla recutita*

German chamomile has been used as an anti-inflammatory and anti-spasmodic for a long time (Ody, 1993). The major component of the flower's essential oil is matricine [(pro-) chamazulene]. Formation of matricine is controlled by a single gene *M*, with the genotype *M_* being free of chamazulene and *mm* containing chamazylene (Fig. 1.2).

Other important compounds in chamomile oil are the sesquiterpene bisaboloids, which produce four different chemotypes based on bisabolol, bisabololoxide A, bisabololoxide B and bisabolonoxide. The precusor of bisabolol is farnesene, which is regulated by gene *L*. In the presence of *L_*, farnesene is converted to bisabolol. Genes *O* and *P* are responsible for bisabololoxide A and B. In the presence of *P_*, bisabololoxide A is formed whereas in the homozygous recessive state, *pp*, bisabolonoxide B is formed.

Bisabolonoxide can be formed only in the presence of bisabololoxide A under the influence of gene *N* (Fig. 1.2; Franz, 1993).

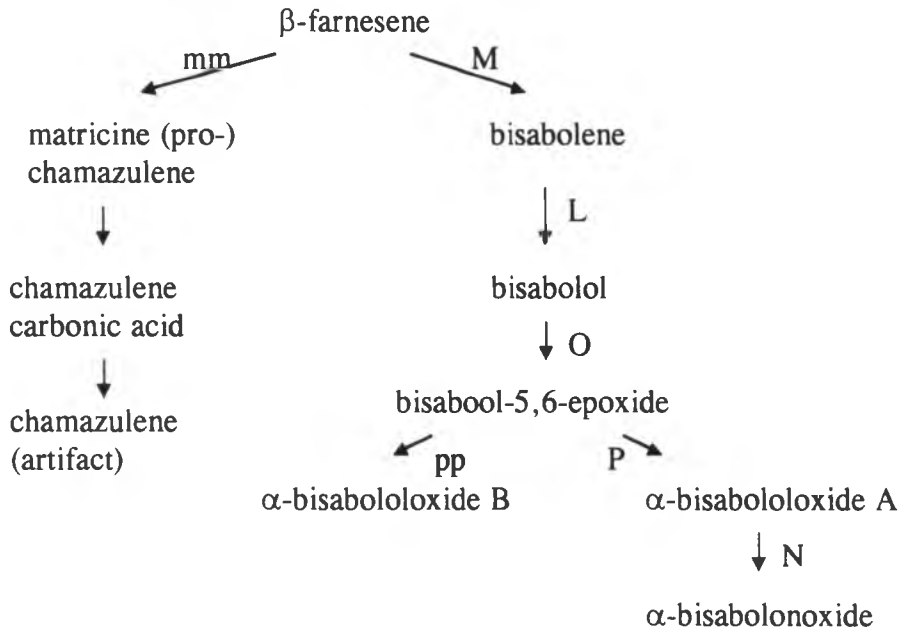

Figure 1.2 Biosynthesis pathway of sesquiterpenes in *Chamomilla recutita*. (Modified from Franz, 1993.)

1.5.2 *Mentha* spp.

The essential oil of mint is considered industrially important as it is used in pharmaceuticals, food flavoring and cosmetics. The biosynthesis and inheritance of monoterpenes in mints, *Mentha* spp., have been studied by Tucker *et al.* (1991). Results of breeding experiments showed that most terpene biosynthesis is due to single chemical

reactions following the one gene-one enzyme hypothesis. There are about eight major genes involve in the production of mint essential oils. The major compounds in mint, carvone and dihydrocarvone, are controlled by gene *C*. Gene *I* regulates the accumulation of linalool whereas gene *Lm* regulates the conversion of limonene to other cyclic monoterpenes. Gene *R* regulates the conversion of the 2-oxygenated and 3-oxygenate ketones to their corresponding alcohols. The accumulation of different compounds is controlled by different group of genes, pulegone and dihydrocarvone by gene *A*, menthone and isomenthone by gene *P*, and piperitone oxide and piperitenone oxide by gene *O*. The esterification of monoterpene alcohols is regulated by gene E (Tucker *et al.*, 1991). Furthermore the genes *A*, *R* and *E* were independently inherited, whereas *P* and *F* were probably linked. There was also evidence that the one gene-one enzyme may not hold in this case, as two different compounds such as dihydrocarvone and carveol could be formed from one precursor, namely carvone. In this example, epistasis must be considered (Fig. 1.3, Table 1.2; Franz, 1993).

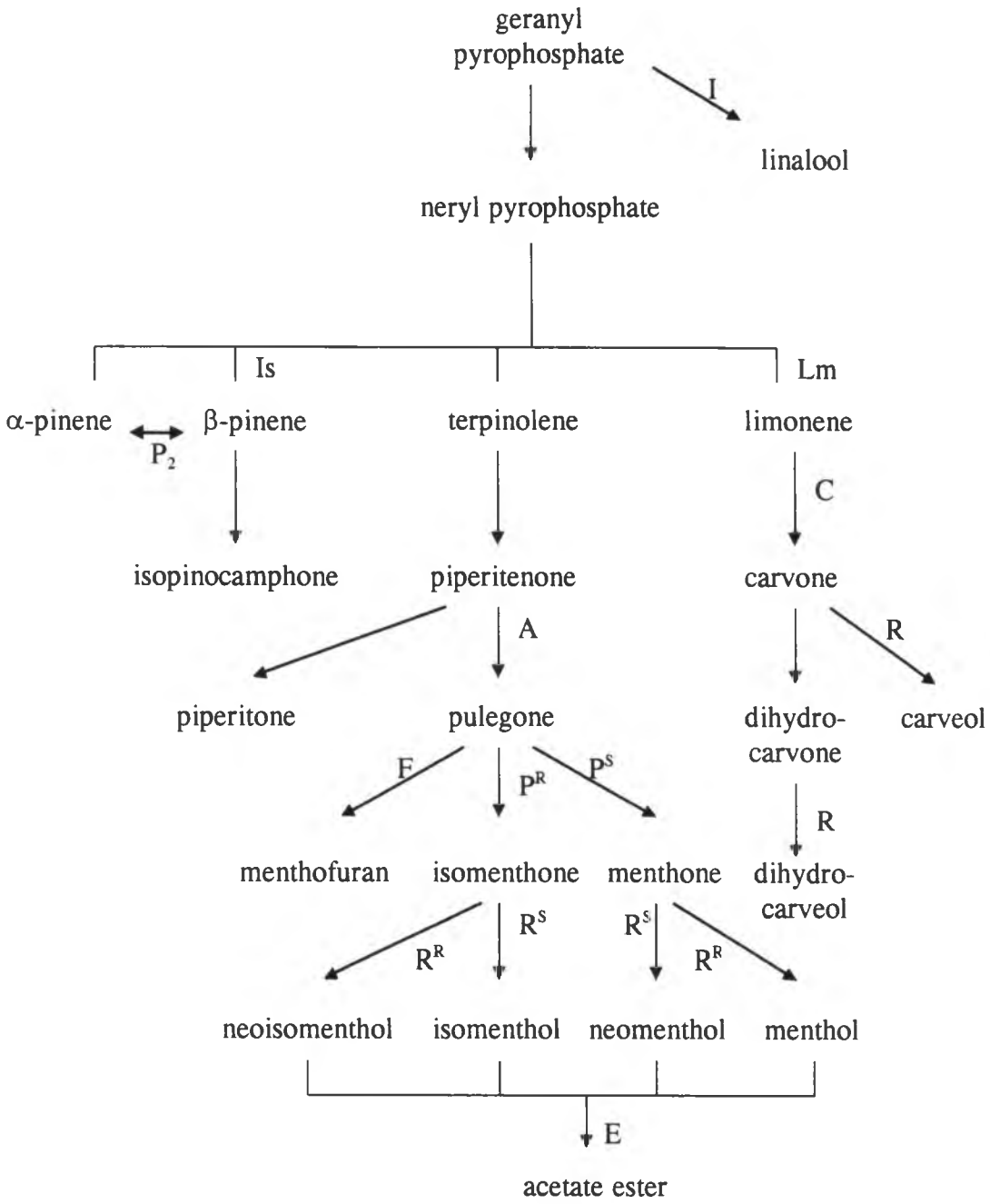

Figure 1.3 Biosynthesis pathway of monoterpenes in *Mentha* species. (Modified from Franz, 1993.)

Table 1.2 Genetic control of chemical constituents in genus *Mentha*. (Modified from Franz, 1993.)

Species	Genotype									Phenotype
	I	Is	Lm	C	A	F	P	R	E	
M. arvensis ssp. *piperascens*	ii	isis	lmlm	cc	AA	FF	PP	R_	(E)	menthol
M. arvensis menthone type	ii	isis	lmlm	cc	AA	FF	PP	rr	(E)	menthone
M. aquatica	ii	isis	lmlm	cc	AA	ff	P/p	R/r	(E)	menthofuran
M. citrata	I_	isis	Lm/lm	C/c	A/a	F/f	P/p	R/r	E_	linalool/ linalyl acetate
isopinocaphone type	ii	Is_	Lm_	C_	-	-	-	-	-	isopinocam-phone
limonene hybrid	ii	isis	Lm_	cc	-	-	-	-	-	limonene
M. spicata (*M. crispa*)	ii	isis	Lm_	C_	-	-	-	-	-	carvone

1.5.3 *Ocimum basilicum*

There are several types of sweet basil, *Ocimum basilicum*, in the family Labiatae (Laminaceae). Each type has a particular odor and is used for food flavoring. Genetics of one variety, *Ocimum basilicum* var. *glabratum*, has been studied (Gupta, 1994). Plant materials were collected from three different sources, France, West Africa and India. Plants from each location were selfed for four consecutive generations to obtain pure lines. Three chemotypes of this species were identified based on the accumulation of estrogole, eugenol or camphor. Although only one gene, *M*, was responsible for biosynthesis, at least three allelic forms of *M* were involved. Allele *M0*, responsible for estrogole, was dominant to the other two alleles, *M1* and *M2* regulating eugenol and camphor production, respectively. Allele *M1* was dominant to *M2* (Fig. 1.4; Gupta, 1994).

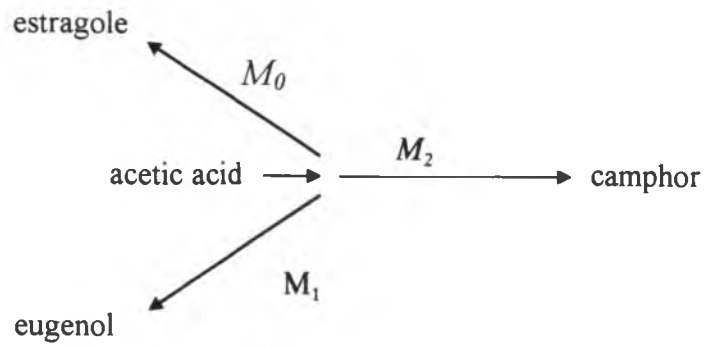

Figure 1.4 Genetic control by one major gene, *M*, of three major chemotypes in *Ocimum basilicum*.

1.5.4 Perilla frutescens

Perilla is grown for perilla oil, generally used in printing-inks and paints (Heywood, 1985). Essential oils from one particular species, *Perilla frutescens*, are used in pharmaceuticals. Genetic control of the chemical composition of volatile compounds in leaves of this species is well understood. Two major genes, *G* and *H*, control the biosynthesis of monoterpenoids. Both genes regulate the conversion of geranylpyrophosphate (GPP), the precursor for monoterpenoid synthesis, to limonene. Genotype *G_* controls the biosynthesis of all kinds of monoterpenoids while *gg*, homozygous recessive state, *gg*, yields phenylpropanoids such as myristicim, dillapoil and elemicin instead of monoterpenoids. In genotypes *H_* with *G_*, cyclic monoterpenoids are formed whereas in the presence of *H_* with *gg*, no monoterpenoids are produced. This means that *gg* is epistatic to gene *H* (Fig. 1.5; Nishizawa *et al.*, 1992).

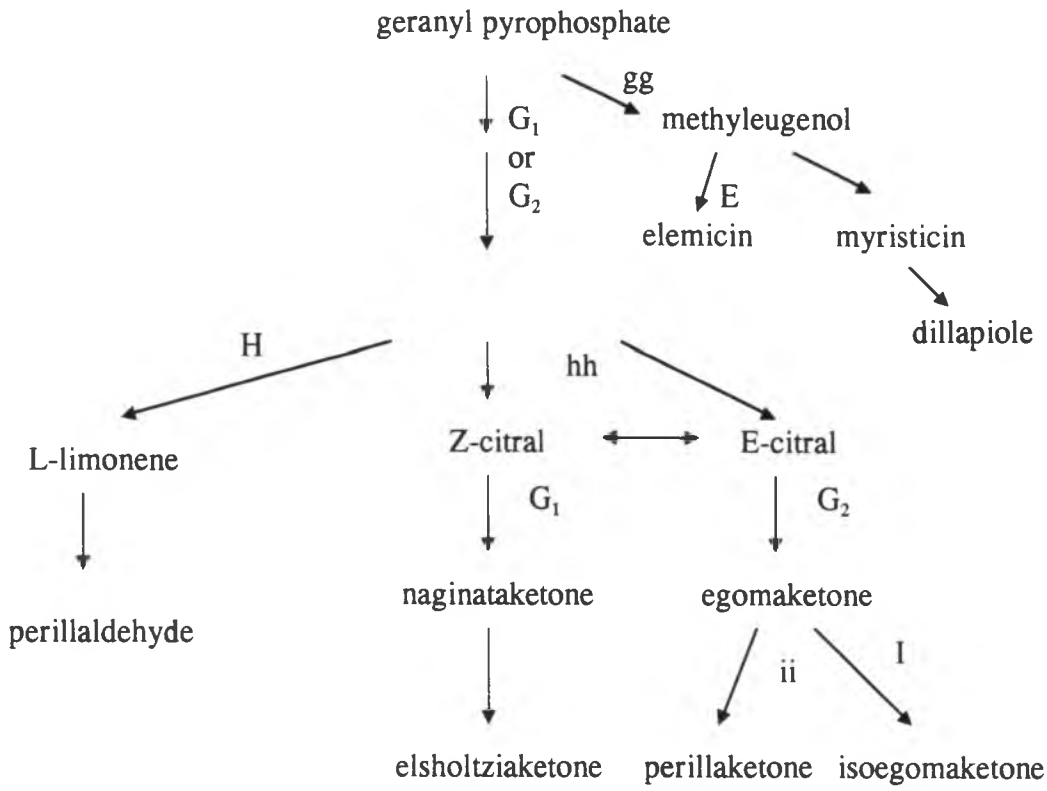

Figure 1.5 Biosynthesis pathways of cyclic monoterpenes in *Perilla frutescens*. (Modified from Nishizawa *et al.*, 1992, and Franz, 1993).

1.5.5 *Oryza sativa*

Rice is one of the most important staple crops in the world. Only a few cultivars of rice are aromatic. Inheritance of rice aroma in six aromatic lines, Della-X2, A-301, Jasmine-85, Amber, PI-457971 and Dragon Eyeball 100, was studied to determine the number of aroma genes and their allelic relationship (Pinson, 1994). Hybrids were made from aromatic lines with five non-aromatic lines. Leaf material from F_1 plants were rated for presence or absence of aroma. F_2 segregation data determined the number of genes and

type of genes that control the aroma character. Leaf and seed samples were tested by smell. Chi-square analysis was employed to compare observed F_2 segregation ratios for aromatic/non-aromatic phenotypes with the F2 segregation ratios expected for one-, two-, or three-gene models.

F_1 plants from the crosses of aromatic lines Jasmine-85, PI-457917, Amber or Dragon Eyeball 100, with non-aromatic lines gave no aroma. This indicated that any aroma gene in these four lines was recessive. Based on F_2 plants results, Amber and Dragon Eyeball 100 contained two recessive aroma genes while Jasmine-85 and PI-457917 each contained a single recessive gene.

2-acetyl-1-pyrroline was a compound found in all rice. Thus, the difference between aromatic and non-aromatic rice is not due to the presence or absence of 2-acetyl-1-pyrroline, but to a different quantity of the chemical in the grain. It was hypothesized that aromatic rice has an enzyme that more effectively catalyzes a step in the biosynthesis of 2-acetyl-1-pyrroline from seed (Pinson, 1994).

1.5.6 *Thymus vulgaris*

An essential oil of common thyme, *Thymus vulgaris*, has been used as an antiseptic in various over-the-counter products such as Vicks Vaporub and Listerine mouthwash. Its essential oil contains about 40 percent thymol (Buchanan, 1995). Dried leaves of thyme are used as seasoning. There are six different chemotypes in this plant species based on accumulation of geraniol, α-terpineol, thujanol-4, linalool, carvacol and thymol as controlled by genes *G*, *A*, *U*, *L*, *C* and *T*, respectively (Fig. 1.6). A stepwise dominance

or epistasis is *G>A>U>L>C>T*. However, the six genes responsible for the chemotypes might not be alleles at the same locus, but rather at five loci (two alleles each) in an epistatic relationship controlling the biosynthetic sequence (Franz, 1993).

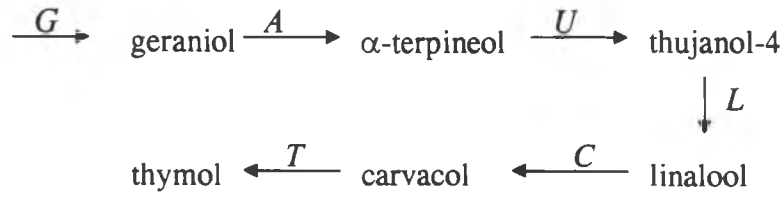

Figure 1.6 Proposed genetic control of monoterpene synthesis in *Thymus vulgaris*.

1.6 Anthurium Genetics and Breeding

1.6.1 Spathe Color

Genetics of the major spathe colors in *Anthurium andraeanum* Hort. are well understood. Kamemoto *et al.* (1988) reported two major genes, *M* and *O*, determined the five major colors red, orange, pink, coral and white. The gene *M* controls the production of cyanidin 3-rutinoside and the gene *O* controls the production of pelargonidin 3-rutinoside. The recessive *oo* is epistatic to *M*, so the genotype for white is *__oo*. Genotypes for the reds and pinks are *M_O_*, and for orange and coral are *mmO_*. The intensity of colors such as red us pink, is affected by the dosage of *M* and *O* alleles. Wannakrairoj and Kamemoto (1990) reported that purple spathe in *A. amnicola* is controlled by gene *P*. Two types of pigments, cyanidin 3-rutinoside and peonidin 3-

rutinoside were present in this species. Gene *P* has an effect on gene *M* and *O* only in the recessive form, *pp*. The spathe is purple when the genotype is *M_O_pp*.

1.6.2 Flower Fragrance

Fragrance genetics in *Anthurium* has not been determined. Studies to date have focussed on observations of scent among species. Croat (1980) reported that many scents are faint while others are very strong. The strong scents are either perfume-like and sweet as for *A. fragrantissimum* Croat, *A. armeniense* Croat, *A. augustispadix* Croat & Baker and *A. hacumense* Engler; or yeasty and foul as for *A. brownii* Mast., *A. salvodorense* Croat and *A. schlechtendalii* Kunth (Table 1.3). In addition, Croat (1980) observed that odor from *Anthurium* was released at different times of the day as well as at different stages of flower development (Table 1.3). Bown (1988) also described some *Anthurium* species that emit scent: *A. amnicola* was minty, *A. armeniense* was lilac-scented and *A. fragrantissimum* was noted to emit fragrance over several weeks although the type of scent was not described.

Table 1.3 Type of scent, stage of development and time of day that fragrant *Anthurium* species emit scent.(Modified from Croat, 1980.)

Species	Scent	Stage of Development	Timing
A. amnicola[a]	minty	-	unknown
A. armeniense	sweet-lilac	pistillate	morning
A. brownii	spoiled fruit	pistillate and staminate	unknown
A. fatoense	sweet	staminate	unknown
A. fragrantissimum	perfume	pistillate and staminate	midday
A. ochranthum	sweet	staminate	unknown
A. hacumense	perfume	pistillate and staminate	unknown
A. salvadorense	sweet-fruity	staminate	unknown
A. schlechtendalii	yeast-like	staminate	unknown
A. standleyi	evergreen	staminate	unknown
A. uplaense	sweet-yeasty	pistillate and staminate	unknown

Note: [a] from Bown, 1988.

1.6.3 Value of Crop and Breeding

Anthurium is an economically important tropical flower traded in the world market. In 1995, anthurium cut flower sales ranked forteenth in the combined Dutch Auctions, with a value of $28.9 million for 36.5 million stems (International Floriculture Quarterly Report, 1996). In Hawaii, the sale value for cut flower was $7 million for 10.9 million stems, and for potted plants was $155,000 for 70,000 pots in 1996 (Hawaii Agriculture Statistics Service, 1997). It has unique and exotic combinations of shape and color of spathe and spadix as well as an excellent shelf life. Anthurium is also popular in the market as blooming potted plant. Breeding for the new cut and potted cultivars has focused on popular and novel colors as well as shape, attractive foliage, long shelf life and disease resistance in the context of other horticulturally desirable traits (Kuehnle *et al.*, 1996).

An *Anthurium* breeding program has been active in the Department of Horticulture, University of Hawaii at Manoa since 1950 (Kamemoto and Kuehnle, 1996). Fragrant anthurium cultivars are not well known and fragrance is not among the characters of the commercial hybrids introduced to the market. Fundamental knowledge of fragrance in *Anthurium* is indeed needed. Studies on scent emission in terms of stage of flower development, time of emission, type of fragrance, inheritance, chemical components and flower histology will provide information useful for breeding programs.

References

Ackerman, D. 1991. *A natural history of the senses*. Vintage Books, A Division of Random House, Inc., New York. 331 pp.

Baumgardt, J. P. 1982. *How to identify flowering plant families*. Timber Press, Portland, Oregon. 269 pp.

Bermadinger-Stabentheiner, E. and A. Stabentheiner. 1995. Dynamics of thermogenesis and structure of epidermal tissues in inflorescences of *Arum maculatum*. *New Phytol.* 131:41-50.

Bown, D. 1988. *Aroids*. Timber Press, Portland, Oregon. 256 pp.

Boyce, P. 1995. Introduction of the Family Araceae. *Curtis's Botanical Magazine* 12: 122-125.

Buchanan, R. 1995. *Taylor's guide to herb*. Houghton Mifflin Co., New York. 453 pp.

Calkin, R. R. and J. S. Jellinek. 1994. *Perfumery: Practice and principles*. John Wiley & Sons, Inc., New York. 287 pp.

Civille, G. V. and J. Close. 1994. Fragrance spectrum descriptive analysis workshop, September 12-14. New Jersey.

Croat, T. B. 1980. Flowering behavior of the neotropical genus *Anthurium* (Araceae). *Amer. J. Bot.* 67: 888-904.

Croteau, R. and F. Karp. 1991. Origin of natural odorants. (*In*) P. M. Muller and D. Lamparsky, eds. *Perfumes: arts, science and technology*. Elsevier Applied Science, New York. pp.101-126.

Dafni, A. 1992. *Pollination Ecology*. IRL Press, New York. 250 pp.

Dobson, H. E. M. 1991. Analysis of flower and pollen volatiles. (*In*) H. F. Linskens and J. F. Jackson, eds. *Modern methods of plant analysis*. Springer-Verlag, Berlin, Germany. pp. 231-251.

Esau, K. 1977. *Anatomy of seed plants*. John Wiley & Sons, Inc., New York. 550 pp.

Forrest, G. W. 1945. Foreward to fragrance. *Amer. Rose Ann.* 30: 66-70.

Fowlis, I. A. 1995. *Gas chromatography*, 2nd ed. John Wiley & Sons Ltd., Chichester, England. 258 pp.

Franz, C. 1993. Genetics. (*In*) R. K. M. Hay and K. P. Svoboda, eds. *Volatile oil crops: their biology, biochemistry and production*. Longman Scientific & Technical, England. pp. 63-96.

Geddes, A. J. 1980. Mass spectrometry. *(In)* S. B. Brown, ed. *An introduction to spectroscopy for biochemists*. Academic Press, New York. pp. 336-392.

Gershenzon, J. and R. Croteau. 1990. Regulation of monoterpene biosynthesis in higher plants. *Recent Adv. Phytochem.* 24:99-160.

Gibbsons, B. 1986. The intimate sense of smell. *Nat. Geo.* 170: 324-360

Gupta, S. C. 1994. Genetic analysis of some chemotypes in *Ocimum basilicum* var *glabratum*. *Plant Breed.* 112: 135-140.

Harborne, J. B. and B. L. Turner. 1984. *Plant chemosystematics*. Academic Press, Orlando, Florida. 562 pp.

Hawaii Agriculture Statistics Service. 1997. Hawaii flowers & nursery products, annual summary. Department of Agriculture, Honolulu, Hawaii. 20 pp.

Hay, R. K. M., and K. P. Svoboda. 1993. Botany. (*In*) R. K. M. Hay and P. G. Waterman, eds. *Volatile oil crops: their biology, biochemistry and production.* Longman Scientific & Technical, England. pp. 5-22.

Heywood, V. H. 1985. *Flowering plants of the world.* Croom Helm Publishers Ltd., Yugoslavia. 335 pp.

International Floriculture Quarterly Report. 1996. *Cut flower sales through the Dutch auctions 1993-1995*. Pathfast Publishing, Essex. 5:12-15.

Jasik, E. R. 1993. Fragrant roses and Henri Delbard. *The Amer. Rose* 32: 12-13.

Jodon, N. E. 1944. The inheritance of flower fragrance and other characters in rice. *J. Amer. Soc. Agron.* 36: 844-848.

Kamemoto, H., R. Y. Iwata and M. Marutani. 1988. Genetics of the major spathe colors in *Anthurium*. College of Tropical Agriculture & Human Resources, University of Hawaii at Manoa, Research Extension Series 056.

Kamemoto, H. and A. R. Kuehnle. 1996. *Breeding anthuriums in Hawaii*. University of Hawaii Press, Honolulu, Hawaii. 132 pp.

Knudsen, J. T. and L. Tollsten. 1991. Floral scent and intrafloral scent differentiation in *Moneses* and *Pyrola* (Pyrolaceae). *Plant Syst. Evol.* 177: 81-91.

Knudsen, J. T., L. Tollsten and L. G. Bergstrom. 1993. Floral scents- a checklist of volatile compounds isolated by head-space techniques. *Phytochem.* 33: 253-280.

Knutson, R. M. 1974. Heat production and temperature regulation in eastern skunk cabbage. *Science* 186:746-747.

Kuehnle, A., J. Halloran, H. Kamemoto, J. Lichty, T. Amore and N. Sugii. 1996. Anthurium cut flower breeding and economics. College of Tropical Agriculture & Human Resources, University of Hawaii at Manoa, Research Extension Series 165.

McLean, F. T. 1933. The inheritance of fragrance in gladiolus species crosses. *The New York Bot. Gard.* 34: 73-80.

Meeuse, B. and S. Morris. 1984. *The sex life of flowers*. Facts on File Publications, New York. 152 pp.

Meilgaard, M., G. V. Civille and B. T. Carr. 1991. *Sensory evaluation techniques*. CRC Press, Inc., Boca Raton, Florida. 281 pp.

Merriam-Webster. 1991. *Webster's ninth new collegiate dictionary*. Merriam-Webster Inc., Springfield, Massachusetts. 1563 pp.

Metcalf, R. L. 1987. Plant volatiles as insect attractants. *CRC Crit. Rev. Plant Sci.* 5: 251-301.

Morris, E. T. 1984. *Fragrance*. Charles Scribner's Sons, New York. 304 pp.

Neuner-Jehle, N. and F. Etzweiler. 1991. The measuring of odors. (*In*) P. M. Muller, and D. Lamparsky, eds. *Perfumes: arts, science and technology*. Elsevier Applied Science, New York. pp. 153-212.

Nishizawa, A., G. Honda and M. Tabata. 1992. Genetic control of the enzymatic formation of cyclic monoterpenoids in *Perilla frutescens*. *Phytochem.* 31: 139-142.

Northen, R. T. 1990. *Home orchid growing*. Simon & Schuster, New York. 376 pp.

Ody, P. 1993. *The complete medicinal herbal*. Dorling Kindersley, New York. 192 pp.

Pinson, S. R. M. 1994. Inheritance of aroma in six rice cultivars. *Crop Sci.* 34: 1151-1157.

Robacker, D. C., B. J. D. Meeuse and E. H. Erickson. 1988. Floral aroma. *BioSci.* 38: 390-398.

Roundnistka, E. 1991. The art of perfumery. (*In*) P. M. Muller and D. Lamparsky, eds. *Perfumes: arts, science and technology*. Elsevier Applied Science, New York. p. 3-48.

Skubatz, H., D. D. Kunkel and B. J. D. Meeuse. 1993. Ultrastructural changes in the appendix of the *Sauromatum guttatum* inflorescence during anthesis. *Sex. Plant Reprod.* 6:153-170.

Skubatz, H., E. R. Svee, B. S. Moore, W. N. Howald, W. Tang and B. J. D. Meeuse. 1995. Oleic acid and it s positional isomer, *cis*-vaccine acid, in the

appendix of *Sauromatum guttatum* during anthesis. *Plant Physiol.* 107:1433-1438.

Smith, B. N. and B. J. D. Meeuse. 1966. Production of volatile amines and skatole at anthesis in some arum lily species. *Plant Physiol.* 41:343-347.

Surburgh, H., M. Guentert, and H. Harder. 1993. Volatile compounds from flowers:Analytical and olfactory aspects. (*In*) R. Teranishi, R. G. Buttery and H. Sugisawa, eds. *Bioactive volatile compounds from plants*. American Chemical Society, Washington DC. pp. 168-186.

Taiz, L. and E. Zeiger. 1991. *Plant physiology*. The Benjamin/Cummings Publishing Company, Inc., Redwood City, California. 559 pp.

Thiboud, M. 1991. Empirical classification of odours. (*In*) P. M. Muller and D. Lamparsky, eds. *Perfumes: arts, science and technology*. Elsevier Applied Science, New York. p. 253-286.

Tucker, A. O., H. Hendrik, R. Bos and D. E. Faribrothers. 1991. The origin of *Mentha x gracilis* (Lamiaceae). II. Essential oils. *Econ. Bot.* 45: 200-215.

Vogel, S. 1990. *The role of scent glands in pollination*. A. A. Balkema, Rotterdam. 202 pp.

Wannakrairoj, S. and H. Kamemoto.1990. Inheritance of purple spathe in *Anthurium*. *J. Amer. Soc. Hort. Sci.* 115: 169-171.

CHAPTER 2

SURVEY OF FRAGRANT *ANTHURIUM* GERMPLASM AT THE UNIVERSITY OF HAWAII AND THE MISSOURI BOTANICAL GARDEN

2.1 Abstract

The total of 147 *Anthurium* species and hybrids at the University of Hawaii and the Missouri Botanical Garden germplasm collections was evaluated for the presence or absence of scent. Type of fragrance, time of emission, daily occurrence and developmental stage of scent emission were recorded along with the color of spathe and spadix. A majority of plants (110/147 or 75%) emitted scent. Fragrance was categorized as citrus, fishy, floral, foul, fruity, menthol, minty, pine, spicy, and sweet. There was no correlation between scent production or scent quality with flower color or botanical section. A plurality of plants emitted scent during the morning (45%) and at the pistillate stage (77%). Fragrance life of unharvested inflorescences varied from 2 to 3 days up to 4 weeks, whereas that of harvested inflorescences was only 1 - 2 days.

2.2 Introduction

Anthuriums are widely available as cut flowers and blooming potted plants. Their exotic flowers with an array of colors and excellent keeping quality on the plant or in the vase make them attractive to the consumer. Addition of novelty such as fragrance would further enhance this ornamental's market desirability (Halloran and Kuehnle, in preparation). Flower fragrance in cultivated varieties of rose, carnation, and gladiolus has been recently reintroduced to increase their popularity among consumers (Barletta, 1995).

Fragrant anthurium hybrids are notably absent in the market. Scent has been reported for eleven species, with quality of scent ranging from perfume-like, sweet, or evergreen to spoiled fruit or foul (Bown, 1988; Croat, 1980). A more extensive survey of fragrance in *Anthurium* species, hybrids and existing cultivars is needed to assess the range of scents available, the time and floral stage of scent emission, and its relationship, if any, to color and environmental conditions. Such knowledge would assist *Anthurium* breeding and marketing programs.

2.3 Material and Methods

2.3.1 Species, Cultivars and Hybrids Survey

A total of 121 *Anthurium* plants, representing 37 different species (40 samples), 23 hybrid accessions, 7 cultivars and 51 hybrid selections under breeding evaluation, was evaluated at the University of Hawaii at Manoa (UH) greenhouses during a three and a half-year period (July 1993 - Feb. 1997) for the presence and nature of flower scent. Inflorescences were observed during the morning (8:00 - 9:30 am), afternoon (1:30 - 3:00 pm) and night (7:30 - 8:30 pm) of clear days. Other data recorded were the floral stage (pistillate and/or staminate) of scent emission, the colors of spathe and spadix, fragrance intensity and the temperature and humidity during observation. Humidity was calculated from temperature of wet and dry bulb.

A total of 26 *Anthurium* plants, representing of 18 species, 3 hybrids and 5 unidentified species, in the aroid collection at the Missouri Botanical Garden, St. Louis, Missouri, was evaluated once in May 1996 during the morning (8:30 - 9:30 am), afternoon (1:30 - 3:00 pm) and night (8:30 - 10:00 pm).

Categories of scent were based on descriptions from Calkin and Jellinek (1994), and Civille and Close (1994). Terms used in describing *Anthurium* scent was determined by the author. More specific descriptors within a category were added when possible. Fragrance intensity was rated on a relative scale of 1 to 3, with 1 being light scent and 3 being strong scent.

2.3.2 Fragrance Life

For potted plants, fragrance life in the greenhouse of one to three unharvested inflorescences of *A. armeniense*, *A. lindenianum*, 'Lady Beth' and hybrid 1213-85 was assessed. Evaluations were made from the time the spathe was fully opened until anthesis. For harvested inflorescences, two to six cut flowers of 'Lady Beth', UH1299 and hybrids 633-41, 649-7, 1195-50 and 1213-22 were observed. Inflorescences were harvested in the morning at the pistillate stage used during commercial harvest, i.e. the spadix is about ¾-mature with receptive stigma (Kamemoto and Kuehnle, 1996) and evaluated in an air-conditioned room at 22-23 C. For unharvested and harvested inflorescences, the presence of fragrance was evaluated daily, three times a day (9:30 am, 1:30 pm and 4:00 pm) until no further fragrance could be detected.

2.4 Results and Discussion

The total number of individual plants observed was 147 (Tables 2.1, 2.2, 2.3 and 2.4). One hundred and ten plants (75%) emitted scent. A plurality of plants (45%) emitted scent during the morning only. Scent from other plants was detected during morning and afternoon hours (28%) and in the afternoon only (6%). Only 16% produced scent morning, afternoon and night.

2.4.1 Species Survey

A total of 52 different *Anthurium* species (58 samples) was evaluated. A majority of 34 species (65%) produced scent. Among the scented species, scent was produced in the morning only; morning, afternoon and night; and morning and afternoon for 35, 38 and 12% of the species respectively (Tables 2.1 and 2.4). None of the species produced scent during the afternoon only. Three species emitted scent at other hours: midday (12:30-1:00 pm) - *A. fragrantissimum*; morning and night - *A. barbadosense*; and night only - *A. jefense*.

Table 2.1 Survey of scent production, fragrance quality and inflorescence colors among *Anthurium* species in the University of Hawaii at Manoa (UH) germplasm collection.

Species (UH accession No.)	Section	Type of fragrance	Stage of development[b]	Time of emission[c]	Spathe/ spadix color
A. amnicola (A417)	Calomystrium	minty	P	morning	lavender/ purple
A. andraeanum (A221)	Calomystrium	floral	P	morning	pink/red
A. antioquiense (A490, A534)[d]	Porphyrochitonium	NF[a]	-	-	lavender/ purple
A. aripoense (A193)	Belolonchium	fishy	P/S	all day	green/green
A. armeniense (A382)	Calomystrium	sweet	P	morning	white/red
A. bakeri (A116)	Porphyrochitonium	NF	-	-	green/green
A. barbadosense (A594)	Porphyrochitonium	foul	P/S	morning, night	green/green
A. bicollectivum (A237)	Porphyrochitonium	foul	P	morning	green/green
A. brownii (A657)	Belolonchium	fishy	P	all day	green/brown
A. cerrobaulense (A332)	Belolonchium	fruity (rotten)	P	morning	green/brown
A. erythrostachyum (A593)	Calomystrium	minty	S	all day	white green/ lavender
A. fatoense (A659)	Pachyneurium	NF	-	-	green/green
A. folsomii (A280)	Porphyrochitonium	fruity	P/S	all day	green/green
A. formosum (A291)	Calomystrium	minty	P	all day	pink/yellow
A. formosum (A507)	Calomystrium	spicy	P	morning	pink/ lavender
A. fragrantissimum (A662)	Porphyrochitonium	floral	P/S	midday	green/white
A. gladiifolium (A317)	Urospadix	fruity (rotten)	P/S	no data	maroon/maroon
A. gracile (A444)	Leptanthurium	floral	S	morning	green/white
A. grande (A373)	Cardiolonchium	NF	-	-	
A. harleyii (A575)	Urospadix	fruity (ripe)	P/S	all day	purple/ purple
A. jefense (A324)	Porphyrochitonium	fruity (melon)	P	night time	green/green
A. lindenianum (A220)	Calomystrium	minty	P/S	day time	white/white
A. nymphaefolium (A213)	Calomystrium	minty	P	morning	white/white
A. ochranthum (A670)	Belolonchium	pine	S	all day	green/yellow
A. pittieri (A269)	Oxycarpium	fishy	P/S	all day	green/green
A. ravenii (A224)	Calomystrium	fishy	P/S	all day	green/yellow

Table 2.1 (continued) Survey of scent production, fragrance quality and inflorescence colors among *Anthurium* species in the University of Hawaii at Manoa (UH) germplasm collection.

Species (UH accession No.)	Section	Type of fragrance	Stage of development[b]	Time of emission[c]	Spathe/ spadix color
A. ravenii (A228)	Calomystrium	fishy	P/S	all day	green/yellow
A. salvadorense	Pachyneurium	NF	-	-	green/white
A. sanctifidense (A503)	Porphyrochitonium	menthol	P	morning	green/white
A. sanctifidense (A592)	Porphyrochitonium	NF	-	-	green/white
A. scherzerianum (A318)	Porphyrochitonium	NF	-	-	red/yellow
A. schlechtendalii (A411)	Pachyneurium	NF	-	-	green/brown
A. scolopendrinum	Leptanthurium	NF	-	-	brown/brown
A. superbum (A488)	Pachynerium	NF	-	-	green/brown
A. standleyi (A658)	Pachyneurium	NF	-	-	green/green
A. trinerve (A238)	Tetraspermium	NF	-	-	white/purple
A. warocqueanum (A101)	Cardiolonchium	NF	-	-	green/green
A. watermaliense (A322)	Pachyneurium	floral (marigold)	P/S	day time	black/black
A. sp. (unknown from Peru) (A596)		citrus (lemongrass)	P/S	no data	green/green
A. sp. (unknown from Panama) (A607)		NF	-	-	green/yellow

[a] NF = non-detectable fragrance.

[b] P = pistillate; S = staminate.

[c] All day = scent detected at 8:00-9:30 am, 1:30-3:00 pm and 7:30-8:30 pm; day time = scent detected at 8:00-9:30 am and 1:30-3:00 pm; morning = scent detected at 8:00-9:30 am; midday = scent detected at 12:30-1:00 pm; night = scent detected at 7:30-8:30 pm.

[d] see chapter 4, chemical analysis of flower cut from A534 and brought to St. John laboratory had a very weak minty scent; no scent was detected in the greenhouse environment.

Table 2.2 Survey of scent production, fragrance quality and inflorescence colors for *Anthurium* hybrids in the University of Hawaii at Manoa (UH) germplasm collection.

Hybrids (UH accession no.)	Type of fragrance	Stage of development[b]	Time of emission[c]	Spathe/ spadix color
A. andraeanum × *A. antioquiense* (A494)	NF[a]	-	-	light red/yellow
[*A. andraeanum* (pink) × *A. antioquiense*] × *A. andraeanum* 'Blushing Bride'(A631)	floral	P	morning	pink/red
{[*A. andraeanum* × *A. antioquiense*] × [*A. andraeanum* (pink) × *A. antioquiense*]} × *A. andraeanum* 'Tagami' (A632)	floral	P	morning	white/red
[*A. andraeanum* (pink) × *A. antioquiense*] × *A. formosum* 'Hilo Hybrid' (A625)	minty	P	morning	pink/lavender
A. antioquiense × *A. amnicola* (A491)	NF	-	-	lavender/purple
A. antioquiense × *A. armeniense* (A628)	sweet	P	day time	pink/lavender
A. crystallinum × *A. armeniense* (A624)	spicy	P	morning	white/purple
A. hookeri × *A.* 'Birdnest' (A630)	sweet	P	morning	white/lavender
A. scherzerianum × *A. antrophyoides* (A535)	sweet similar to A382	P	morning	pink/lavender
[*A. veitchii* × *A. andraeanum* 'Bob Wilson Red'] × *A. formosum* 'Hilo Hybrid' (A629)	minty	P	morning	red/yellow
'Blush Tulip' (A568)	floral	P	day time	white/red
'Congo' (A440)	minty	P	afternoon	white/light purple
'Fujii Light Pink' (A646)	fruity	P	day time	pink/yellow
'Lady Beth' (A602)	sweet, floral	P	morning	pink/lavender
'Lady Jane' (A558-4)	floral	P	day time	light red/yellow
'Leilani' (A563)	minty	P	day time	lavender/lavender
'Pink Aristocrat' (A566)	minty	P	day time	pink/red
'Shipman Pink' (A601)	sweet, minty	P	day time	pink/yellow
'Shiroma's Splash' (A452)	floral	P	morning	pink obake/red
unnamed brown (A672)	floral	P	day time	brown/green
unnamed green (A675)	sweet, floral	P	afternoon	green/brown
unnamed pink (A626)	sweet, floral similar to A382	P	day time	light pink/pink
unnamed red tulip (A680)	minty	P	morning	red/red

[a] NF = non-detectable fragrance.

[b] P = pistillate; S = staminate.

[c] All day = scent detected at 8:00-9:30 am, 1:30-3:00 pm and 7:30-8:30 pm; day time = scent detected at 8:00-9:30 am and 1:30-3:00 pm; morning = scent detected at 8:00-9:30 am; midday = scent detected at 12:30-1:00 pm; night = scent detected at 7:30-8:30 pm.

Table 2.3 Survey of scent production, fragrance quality and inflorescence colors of University of Hawaii-originated *Anthurium* hybrids, breeding lines and selections.

Cultivar, cross no. or selection no.	Type of fragrance	Stage of development[b]	Time of emission[c]	Spathe/ spadix color
'ARCS'	minty	P	day time	lavender/purple
'Chamelian'	floral	P	morning	white-green/green
'Hokulea'	minty	P	morning	pink/lavender
'Manoa Mist'	floral	P	morning	white/yellow
'Paradise Pink'	floral	P	morning	pink/yellow
'Satan'	pine	P	all day	light purple/ green
'Trinidad'	minty	P	afternoon	pink/purple
572-23	minty	P	morning	purple/purple
633-41	minty	P	all day	white/white
649-2, -7	sweet, floral	P	morning	white/red
768-7, -26, -27, -47	sweet, floral	P	day time	pink/orange
1061-4	sweet	P	day time	white/red
1061-11	floral, sweet	P	day time	white/lavender
1081-1	floral	P/S	all day	green/white
1131-7	floral	P	day time	red/yellow
1147	fruity (melon)	P	morning	green/brown
UH585	minty	P	morning	orange/yellow
UH1131	floral	P	morning	red/red
UH1141	minty, spicy	P	day time	pink/pink
UH1198	minty	P	morning	red/red
UH1207	minty	P	morning	purple/purple
UH1211	floral	P	morning	red obake/red
UH1228	minty, floral	P	day time	light red/lavender
UH1241	NF[a]	-	-	green/green
UH1245	floral	P	day time	red/yellow
UH1246	floral	P	day time	coral/orange
UH1269	NF	-	-	light red/orange
UH1299	sweet, floral	P	all day	pink/pink
UH1272	minty	P	morning	white-green/orange
UH1311	floral	P	morning	red/red
UH1321	floral	P	day time	pink/green
UH1326	NF	-	-	white/white

Table 2.3 (continued) Survey of scent production, fragrance quality and inflorescence colors of University of Hawaii-originated *Anthurium* hybrids, breeding lines and selections.

Cultivar, cross no. or selection no.	Type of fragrance	Stage of development[b]	Time of emission[c]	Spathe/ spadix color
UH1332	floral	P	day time	pink obake/green
UH1349	floral	P	afternoon	white/orange
UH1394	NF	-	-	pink/pink
UH1402	floral	P	day time	pink/pink
UH1403	minty	P	afternoon	light red/lavender
UH1404	minty	P	afternoon	white/red
UH1450	floral	P	day time	white/orange
UH1461	sweet	P/ S	day time	pink obake/ lavender
UH1462	floral	P	day time	pink/orange
UH1463	NF	-	-	lavender/purple
UH1465	sweet	P	day time	red/green
UH1469	NF	-	-	purple/purple
UH1472	minty	P	morning	white-green/orange
UH1482	floral	P	morning	red/yellow
UH1534	NF	-	-	purple/purple
UH1553	NF	-	-	pink/lavender
UH1584	floral	P	morning	red/yellow
UH1635	floral	P	afternoon	red/yellow
UH1644	NF	-	-	purple/purple
UH1679	floral	P	morning	red/yellow
UH1718	minty	P	morning	light purple/purple
UH1742	NF	-	-	red/yellow
UH1750	NF	-	-	red/yellow
UH1754	NF	-	-	dark purple/red
UH1756	floral	P	morning	green-red/yellow
UH1761	NF	-	-	pink/red

[a] NF = non-detectable fragrance.

[b] P = pistillate; S = staminate.

[c] All day = scent detected at 8:00-9:30 am, 1:30-3:00 pm and 7:30-8:30 pm; day time = scent detected at 8:00-9:30 am and 1:30-3:00 pm; morning = scent detected at 8:00-9:30 am; midday = scent detected at 12:30-1:00 pm; night = scent detected at 7:30-8:30 pm.

Table 2.4 Survey of scent production, fragrance quality and inflorescence colors of 23 *Anthurium* species and 3 hybrids at the Missouri Botanical Garden (MBG) germplasm collection.

Species (MBG accession no.)	Section	Type of fragrance	Stage of development[b]	Time of emission[c]	Spathe/ spadix color
A. armeniense (63434)[d]	Calomystrium	NF[a]	-	-	white/ red
A. atropurpureum v. *arenicolum* (53698)	Pachyneurium	fruity (rotten)	S	morning	reddish green/ brown
A. barclayanum (50712)	Pachyneurium	fruity (rotten)	S	all day	green/ lavender green
A. bonplandii (69761)	Pachyneurium	foul	P	all day	green/ green
A. chiapasense ssp. *tlaxiacense* (46126)	Belolonchium	foul	S	morning	green/ brown
A. cogolloanum	unidentified	NF	-	-	green/ pink
A. croatii (51656)	Dactylophyllum	fruity (rotten)	P	day time	green/ green
A. digitatum (54378)	Dactylophyllum	fruity (rotten)	P	morning	green/ lavender
A. ferrienense (57160)	Calomystrium	sweet	P	morning	white/ red
A. lancetillense	Belolonchium	fruity (rotten)	S	morning	green/ dark purple
A. nymphaefolium (45022)	Calomystrium	minty	P	morning	white/ white
A. ochranthum (69861)	Belolonchium	pine	S	all day	green/ yellow
A. plowmanii (53563)	Pachyneurium	fruity (rotten)	S	day time	reddish green/ brown green
A. prolatum (76532)	Pachyneurium	NF	-	-	green/ brown
A. radicans (76139)	Chamaerepium	NF	-	-	green/maroon
A. solitarium (61798)	Pachyneurium	foul	P	all day	green/ lavender
A. subsignatum (49788)	Semaeophyllium	floral (marigold)	P	all day	green/ yellow
A. tarapotense (58115)	Pachyneurium	NF	-	-	green purple/ purple
A. willifordii (73936)	Pachyneurium	NF	-	-	maroon/red

Table 2.4 (continued) Survey of scent production, fragrance quality and inflorescence colors of 23 *Anthurium* species and 3 hybrids at Missouri Botanical Garden (MBG) germplasm collection.

Species (MBG accession no.)	Section	Type of fragrance	Stage of development[b]	Time of emission[c]	Spathe/ spadix color
(*A. crystallinum* x *A. papillilaminum*)	-	NF	-	-	green/maroon
(*A. papillilaminum* x *A. crystallinum*) (TC1066)	-	NF	-	-	green/green
A. radicans hybrid (75496)	-	floral (green)	P	morning	greenish purple/ reddish purple
unidentified (74030, No. 1)	-	minty	P	morning	white/ lavender
unidentified (74030, No. 2)	-	minty	S	day time	white/ lavender
unidentified (75522)	-	rotten fruit	S	all day	green/brown
unidentified (76360)	-	minty	P	morning	green/ yellow

[a] NF = non-detectable fragrance.

[b] P = pistillate; S = staminate.

[c] All day = scent detected at 8:00-9:30 am, 1:30-3:00 pm and 7:30-8:30 pm; day time = scent detected at 8:00-9:30 am and 1:30-3:00 pm; morning = scent detected at 8:00-9:30 am; midday = scent detected at 12:30-1:00 pm; night = scent detected at 7:30-8:30 pm.

[d] young, pre-pistillate inflorescence.

Fragrance was detected for 47% of the scented species at the pistillate stage, for 21% at the staminate stage and for 32% during both pistillate and staminate stages. A young, pre-pistillate-stage inflorescence of *A. armeniense* (MBG no. 63434, Table 2.4) was scentless, yet this species is recognized to emit a "perfume-like" (Croat, 1980) and "delightful" (Kamemoto and Kuehnle, 1996) fragrance. A more mature pistillate-stage inflorescence of *A. armeniense* (UH no. A382, Table 2.1) indeed released a very sweet fragrance.

Two different plant accessions were observed for each of six species: *A. armeniense*, *A. formosum*, *A. nymphaefolium*, *A. ochranthum*, *A. ravenii* and *A. sanctifidense* (Tables 2.1 and 2.4). Type of scent and time of emission were similar for each pair of *A. nymphaefolium*, *A. ochranthum* and *A. ravenii*. In contrast, *A. formosum*, accessions A291 and A507 differed in type and time of fragrance, with A291 being minty all day long and A507 being spicy and emitting only in the morning. *A. sanctifidense* A503 yielded menthol fragrance while no scent could be detected from A592. These discrepancies might be due to variation in essential components within the species, as described for sweet basil, *Ocimum basilicum* (Grayer *et al.*, 1996).

2.4.2 Hybrids Survey

A total of 81 hybrids was evaluated, with 81% producing scent. Time of scent emission varied as follows: 45% of the plants were scented in the morning only, 38% were scented during the daytime, 11% were scented all day and 6% were scented in the afternoon only. Eighty percent of the fragrant hybrids emitted scent at the pistillate stage only and 20% produced scent at both pistillate and staminate stages. None released scent at only the staminate stage.

2.4.3 Type of Fragrance

Ten types of scent were detected among species. These were broadly classified as citrus, fishy, floral, foul, fruity, menthol, minty, pine, spicy, and sweet (Tables 2.1 and 2.4). Some categories included more specific scents such as lemongrass (citrus), melon, ripe or rotten (fruity) and marigold or green (floral).

Scents varied widely within botanical sections. For example, scents among member species of section Belolonchium ranged from unpleasant- such as fishy or rotten fruit- to sweet pine. Section Calomystrium produced generally very pleasant fragrance-such as sweet floral or minty- but included the fishy smell of *A. ravenii*. In section Porphyrochitonium, both pleasant and unpleasant types could also be found (Tables 2.1 and 2.4). While flowers may emit different scents to attract different groups of pollinators (Proctor *et al.*, 1996), the pollinators for *Anthurium* are poorly described (T. B. Croat, 1996 personal communication). Scented *Anthurium* displayed a

wide variation in spathe colors, including black, brown, green, lavender, maroon, pink, purple, red and white. Specific scent types were not associated with particular spathe colors.

Most of the observed hybrids (Tables 2.2, 2.3 and 2.5) represent sections Calomystrium and Porphyrochitonium. These two sections contribute valuable species for use in cut-flower breeding, including the fragrant *A. amnicola* (Fig. 2.1), *A. armeniense* (Fig. 2.2) and *A. lindenianum* (Fig. 2.3). The most common fragrances found amongst 81 hybrids having at least one of those species as a parent were floral (61%) and minty (33%). Some hybrids produced a mixture between floral and minty, described as sweet and floral or minty and sweet. Examples include, 'Lady Beth', 'Shipman Pink', A626, A675, 649-2, 649-7, 768-7, 768-26, 768-27, 768-47, 1061-11, UH1141 and UH1299.

Table 2.5 Parental species of fragrant University of Hawaii-originated *Anthurium* hybrids, breeding lines and selections.

Parental species	Section	Fragrance of University of Hawaii-originated hybrids, breeding line or selections	
A. amnicola (minty)	Calomystrium	minty	ARCS, 572-23, UH1272 UH1403
A. antioquiense (non-fragrant)[a]	Porphyrochitonium	floral	UH1245, UH1311, UH1332, UH1402, UH1548, UH1679
		minty	UH1272
		sweet	UH1461, UH1465
		sweet, floral	768-7, 768-26, 768-27, 768-47, UH1299
A. armeniense (sweet)	Calomystrium	sweet, floral	649-7
A. formosum (minty, spicy)	Calomystrium	minty	572-23
A. lindenianum (minty)	Calomystrium	floral	UH1450, UH1462
		minty	'Trinidad', 633-41, UH1272
		pine	Satan

[a] This was later determined to be weakly scented in the laboratory environment. See chapter 4, chemical analysis.

Section	Calomystrium
Fragrance	Minty
Time	Morning
Stage	Pistillate

Figure 2.1 *A. amnicola* (A417)

Section	Calomystrium
Fragrance	Sweet Floral
Time	Morning
Stage	Pistillate

Figure 2.2 *A. armeniense* (A382)

Section	Calomystrium
Fragrance	Minty
Time	Morning
Stage	All day

Figure 2.3 *A. lindenianum* (A220-2)

2.4.4 Environmental Factors

Conditions of observation at the University of Hawaii were: morning - 74% to 100% RH, 17 to 25 C; afternoon - 65% to 80% RH, 28 to 30 C; night - 85% to 90% RH, 20 to 24 C. The most difficult time to detect scent by sniffing flowers was at 100 % RH, especially at temperatures less than 18 C. This might be due to evaporable compounds being saturated around the inflorescence as well as to a decrease in fragrance production under these conditions. There was a negative association between fragrance intensity and humidity (correlation coefficient, r = -0.786, Fig. 2.4) and a positive association between fragrance intensity and temperature (correlation coefficient, r = 0.75, Fig. 2.5).

Four species obtained by UH from Missouri Botanical Garden, *A. fatoense*, *A. salvadorense*, *A. schlechtendalii* and *A. standleyi*, were found scentless, contrary to a previous report (Croat, 1980; Table 2.1). Although this disparity may be due to different accessions, it also may be due to environmental factors. For example, the amount of volatile compounds released from the four accessions may have been insufficient and/or undetectable by the human nose under temperature and humidity conditions of the Hawaii greenhouse. Indirect supporting evidence comes from a progeny of 'Ellison Onizuka' and *A. armeniense*, 1213-85, with light fragrance detected at 100% RH, 18 C and strong fragrance was detected at 80% RH, 22 C. Temperature influenced monoterpene emission in slash pine (Tingey *et al.*, 1979) and in peppermint (Burbott and Loomis, 1967). Monoterpene was synthesized in greater amount when the temperature increased.

Anecdotal evidence in rose also indicates that humidity and temperature are involved in fragrance emission (Allen, 1980; Bouquet, 1968; Carruth, 1992; Harkness, 1992). Based on reported correlations between scent and humidity and temperature, and on suggestions from studies of other crops, the effect of these factors on *Anthurium* fragrance emission should be further tested.

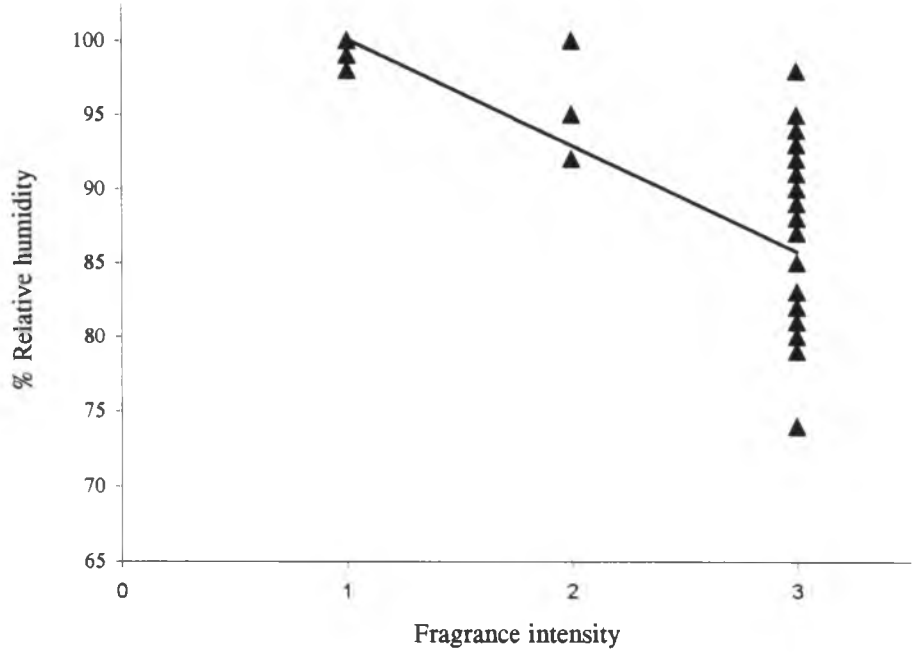

Figure 2.4 Correlation between fragrance intensity and relative humidity for scented *Anthurium* species and hybrids. 1= light scent, 2 = medium scent and 3 = strong scent. (r = -0.786)

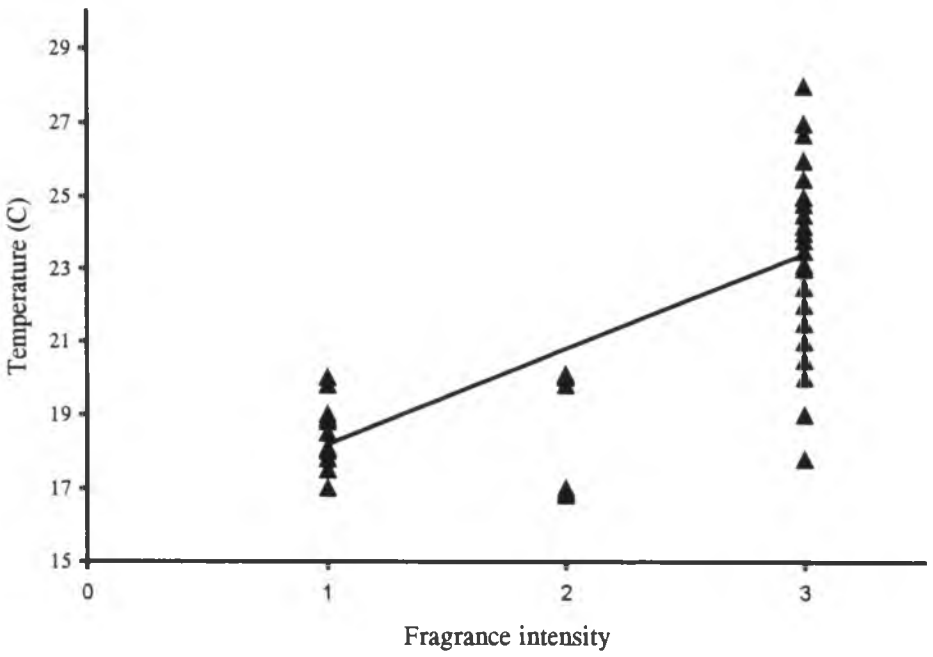

Figure 2.5 Correlation between fragrance intensity and temperature for scented *Anthurium* species and hybrids. 1= light scent, 2 = medium scent and 3 = strong scent. (r = 0.75)

2.4.5. Fragrance Life

Fragrance life for unharvested inflorescences ranged from 3 to 20 days (Table 2.6). The long fragrance life of *A. armeniense* and *A. lindenianum* makes them attractive for a breeding program. In this experiment, *A. armeniense* was a male parent for 1213-85, which also had a long fragrance life.

Fragrance life of harvested inflorescences was short, only 1 to 2 days (Table 2.7). The shortest fragrance life was found in 1195-50, lasting only for the morning. Fragrance of 'Lady Beth' and 633-41 lasted until early afternoon. Fragrance life of 649-7, 1213-22 and UH1299 lasted for two days. Harvesting inflorescences decreased their fragrance life. In 'Lady Beth' fragrance life of unharvested inflorescences lasted for 3-5 days and of harvested flowers lasted for less than one day.

Table 2.6 Fragrance life of unharvested *Anthurium* inflorescences.

Species, cultivar or hybrid	No. of flowers observed	Type of fragrance	Fragrance life (days)
A. armeniense (A613)	2	sweet	10-14
A. lindenianum (A220-2)	1	minty	20
'Lady Beth'	3	minty	3-5
'Ellison Onizuka' × *A. armeniense* (1213-85)	1	sweet, floral	18

Table 2.7 Fragrance life of harvested *Anthurium* inflorescences.

Species or cultivar	No. flowers observed	Type of fragrance	Fragrance life after harvest (days)
'Lady Beth'	3	minty	1[a]
633-41	1	minty	1[a]
649-7	2	sweet, floral	2
1195-50	1	minty	1[b]
1213-22	1	floral	2
UH1299	6	sweet, floral	2

[a] fragrance could be detected only at 9:30 am. and 1:30 pm.

[b] fragrance could be detected only at 9:30 am.

2.5 Summary

In this survey, only 5% of about 1000 known *Anthurium* species were evaluated. Scent production in *Anthurium* is not yet well known. Further observations, especially in natural habitats, might provide more information about *Anthurium* phylogeny and relationships between scent and type of pollinator. While different categories of scent were identified in this study, only those species or hybrids that release pleasant fragrance, for example *A. armeniense*, would be desirable in a breeding program. Results of the fragrance life study with unharvested and harvested inflorescences suggest that breeding for fragrance should focus on potted plants. Further study on the effect of the environment on fragrance emission is needed in order

to develop recommendations for cultural practice and marketing of quality fragrant plants.

References

Allen, E. F. 1980. Rose scent- A quantitative study. *The Rose Ann.* pp. 58-61.

Barletta, A. 1995. Scent makes a come back. *FloraCul. Inter.* pp. 23-25.

Bouquet, A. 1968. Perfumes of the rose. *Amer. Rose Ann.* 53: 107-111.

Bown, D. 1988. *Aroids*. Timber Press, Portland, Oregon. 256 pp.

Burbott, A. J. and W. D. Loomis. 1967. Effects of light and temperature on the monoterpenes of peppermint. *Plant Physiol.* 42: 20-28.

Calkin, R. R. and J. S. Jellinek. 1994. *Perfumery: Practice and principles*. John Wiley & Sons, Inc. New York. 287 pp.

Carruth, T. 1992. The fickle finger of fragrance. *Amer. Rose Ann.* 77: 24-25.

Civille, G. V. and J. Close. 1994. Fragrance spectrum descriptive analysis workshop, September 12-14, New Jersey.

Croat, T. B. 1980. Flowering behavior of the neotropical genus *Anthurium* (Araceae). *Amer. J. Bot.* 67: 888-904.

Grayer, R. J., G. C. Kite, F. J. Goldstone, S. E. Bryan, A. Paton and E. Putievsky. 1996. Infraspecific taxonomy and essential oil chemotypes in sweet basil, *Ocimum basilicum. Phytochem.* 43: 1033-1039.

Harkness, P. 1992. Roses for noses. *Amer. Rose Ann.* 77: 15-17.

Kamemoto, H. and A. R. Kuehnle. 1996. *Breeding anthuriums in Hawaii*. University of Hawaii Press, Honolulu, Hawaii. 132 pp.

Proctor, M., P. Yeo, and A. Lack. 1996. *The natural history of pollination*. Timber Press, Portland, Oregon. 479 pp.

Tingey, D. T., M. Manning, L. C. Grothaus and W. F. Burns. 1979. The influence of light and temperature on isoprene emission rates from live oak. *Physiol. Plant* 47: 112-118.

CHAPTER 3

INHERITANCE OF SCENT IN *ANTHURIUM*: A PRELIMINARY STUDY

3.1 Abstract

F_1 progenies of crosses between fragrant × fragrant and non-fragrant × fragrant parents were studied in order to determine whether a single gene or more than one gene governed this character. Presence and absence of scent was detected using the author's nose. Progenies from 24 crosses were tested for a single dominant or recessive trait. Chi-square analysis was used for testing goodness of fit. Progeny segregated for fragrance quality and included non-parental scents. None of the progenies corresponded to an expected segregation ratio. Results indicated that multiple genes likely govern the presence of fragrance trait in *Anthurium*.

3.2 Introduction

Fragrance is one important attribute in florist crop aesthetics. Rose and carnation, for example, are well known for pleasant scents that enhance their market value. Introgression of fragrance from species into cultivated varieties was recently reported for *Gladiolus* (Anderton and Park, 1989) and begonia (Jackson & Perkins, 1996, catalogue).

However, genetics of flower fragrance is documented only for *Gladiolus* (McLean, 1933, 1938) and rice (Jodon, 1944).

Anthurium is an important cut flower in the world market. Introducing fragrance into *Anthurium* would be expected to benefit the anthurium flower industry. Eleven species of *Anthurium* are known to be fragrant (Bown, 1988; Croat, 1980). Additional 37 scented *Anthurium* species are described in Chapter 2. While fragrance has been detected among offspring from crosses made between fragrant and non-fragrant parents (Kamemoto and Kuehnle, 1996), the inheritance of fragrance is unknown.

One objective of this study was to determine the number of genes controlling the presence of fragrance in *Anthurium*. Two hypotheses were proposed for testing by segregation ratios.

1) Scent is governed by a single dominant or recessive gene.

The expected outcome for dominance in F_1 progenies from fragrant × non-fragrant parents would be all fragrant or a 1 : 1 segregation ratio. Conversely, F_1 progenies would be either all non-fragrant or would segregate in a 1 : 1 ratio for a recessive trait.

2) scent is governed by more than one gene, i.e. multiple loci.

The expected outcome for F_1 progenies from fragrant × non-fragrant parents would be lack of correspondence to a segregation ratio by Chi-square analysis. A second objective was to identify parental plants that may be employed as sources of fragrance for future breeding.

Results indicate that multiple genes likely govern the fragrance trait in *Anthurium*. Further study or use of another approach is needed to assess fragrance genetics.

3.3 Material and Methods

A total of 44 crosses between fragrant and non-fragrant or fragrant parents was made in 1992- 1993 (Appendix 1) at the Horticulture Department Research Facility, University of Hawaii at Manoa. Pollen from selected plants was transferred to selected receptive spadices by hand during 8:00-9:00 am. Each cross was labelled. Each spathe with its pollinated spadix was then covered with a plastic bag for three days to avoid any loss of pollen that might occur during rainfall and overhead irrigation. After about six months, mature berries were collected and then squashed in water to separate the mesocarp and seed. Clean seeds were scattered on a medium of finely shredded tree-fern fiber layered 5 cm thick, on top of 2.5 cm (1-in) basalt in 12.5-cm (5-in) pots and kept in a screenhouse under 80% shade. Four to six months after sowing, germinated seedlings were transplanted about 5 cm apart in flats containing a mixture of composted redwood and 0.5-cm perlite (1:1 ratio). Six to eight months after transplanting to flats, seedlings were transplanted into 15-cm (6-in) plastic pots in a medium of composted redwood and 1-cm perlite (3:1 ratio). Evaluations commenced upon flowering 2 - 2.5 years after pollination (generally in early 1995).

Individual plants were examined in the morning, afternoon and night for the type and the presence or absence of flower scent. Each inflorescence was evaluated at its pistillate and staminate stages. Fragrance emission was detected by the author's nose. Plants were scored as fragrant if at least one inflorescence produced detectable scent. Colors of the spadix and fully expanded spathe were also recorded. Each new

inflorescence was evaluated during a 12- to 18-month period, with the number evaluated per progeny plant varying from one to 8 inflorescences. Due to loss of several populations from bacterial blight disease, twenty-five of the forty-four crosses were available for genetic study (Table 3.1). Among these, three populations were from fragrant × fragrant plants (cross numbers 1180, 1181 and 1216). The remainders were crosses between non-fragrant × fragrant plants. Chi-square analysis (Srb *et al.*, 1965) of progeny data tested the possibility of fitted ratios.

Table 3.1 *Anthurium* crosses from 1992-1993 available for genetic study of fragrance.

Cross	Female			Male		
no.	Designation	Color	Fragrance[a]	Designation[b]	Color	Fragrance[a]
1172	UH585	orange	NF	A220-2	white	F
1180	'Trinidad'	pink	F	A220-2	white	F
1181	'Trinidad'	pink	F	A213-2	white	F
1182	UH566	dark pink	NF	A220-2	white	F
1183	A38	red	NF	A220-2	white	F
1184	692-48	white	NF	A220-2	white	F
1185	UH818	pink	NF	A220-2	white	F
1188	A 558		NF	A220-2	white	F
1195	A494	pink	NF	RS1361-1	white	F
1196	UH585	orange	NF	A220-2	white	F
1197	UH186	pink	NF	A220-2	white	F
1198	UH185	red	NF	A220-2	white	F
1200	383	red	NF	A220-2	white	F
1201	A99	red	NF	A212-2	white	F
1202	'Kozohara'	red	NF	A220-2	white	F
1203	'Paradise Pink'	pink	NF	A170	white	F
1204	'Fujii Light Pink'	pink	NF	RS1316-1	white	F
1205	A38	red	NF	A220-2	white	F
1206	A167	orange	NF	A212	white	F
1207	A67	red	NF	A220-2	white	F
1208	A99	red	NF	A212	white	F
1210	'Marian Seefurth'	pink	NF	A220-2	white	F
1212	A167-2	red	NF	A220-2	white	F
1213	'Ellison Onizuka'	white	NF	A382	white	F
1216	649-7[b]	white	F	UH1131	red	F

a. F = fragrant plant; NF = non-fragrant plant

b. A212-2 = *A. hoffmanii*; A213-2 = *A. nymphaefolium*; A220-2 = *A. lindenianum*; A382 = *A. armeniense*; RS1361-1 = *A. lindenianum* (A170) × *A. lindenianum* (A220); 649-7 = 'Manoa Mist' × *A. armeniense* (A382)

3.4 Results and Discussion

The number of progenies from each of 24 crosses that flowers were evaluated are listed in Table 3.2. Progenies from cross number 1181 ('Trinidad' × *A. nymphaefolium*) failed to flower by the end of June 1995, 36 months after pollination. Moreover, the plants were very tall due to long internodes, collapsing on the bench. Therefore, the cross was discarded at the end of June 1995 and eliminated from analysis.

Evaluation of first generation progenies from 22 crosses between non-fragrant × fragrant parents showed none to be uniformly fragrant or non-fragrant, with segregation for presence of scent apparent in all populations (Table 3.2). Thus, no fragrant parents carried a single homozygous dominant or recessive gene governing scent. None of the test ratios fit the expected ratios of 1 : 0 or 0 : 1 for fragrant : non-fragrant.

Moreover, no parental genotypes appeared heterozygous for a single gene for fragrance, as the 1 : 1 ratio of non-fragrant and fragrant progenies was not observed. Only one cross, number 1185, gave the greatest possibility (*P* value), $P>0.95$ for goodness of fit (Table 3.3) but it had a small population size (n=10). The other crosses did not show any significance for this test (Table 3.3). The fragrant parent in this cross, *A. lindenianum*, was used as a parental plant for 15 other crosses (Table 3.4) yet none showed a similar segregation ratio. These results taken together suggest lack of single gene inheritance for scent.

Table 3.2 Number of fragrant and non-fragrant progenies in each *Anthurium* cross population and number culled due to the bacterial blight.

Cross no.	Total	Flowered	Fragrant (%)	Non-fragrant (%)	Bacterial blight[a]
1172	22	17	11 (64.7)	6 (35.3)	4
1180	123	81	67 (82.7)	14 (17.3)	41
1181*[b]	95	7	2 (28.6)	5 (71.4)	4
1182	37	19	17 (89.5)	2 (10.5)	14
1183	7	5	4 (80.0)	1 (20.0)	2
1184	48	9	4 (44.4)	5 (55.6)	39
1185	23	10	5 (50.0)	5 (50.0)	12
1188	95	77	54 (70.1)	23 (29.9)	8
1195	116	86	31 (36.0)	55 (64.0)	15
1196	92	46	27 (58.7)	19 (41.3)	45
1197	9	8	7 (87.5)	1 (12.5)	1
1198	38	25	20 (80.0)	5 (20.0)	13
1200	43	21	14 (66.7)	7 (33.3)	22
1201	42	24	2 (8.3)	22 (91.7)	18
1202	21	13	8 (61.5)	5 (38.5)	8
1203	83	39	27 (69.2)	12 (30.8)	44
1204	101	78	46 (59.0)	32 (41.0)	23
1205	84	35	17 (48.6)	18 (51.4)	49
1206	84	49	15 (30.6)	34 (69.4)	35
1207	61	38	24 (63.2)	14 (36.8)	23
1208	44	21	8 (38.1)	13 (61.9)	23
1210	40	29	10 (34.5)	19 (65.5)	11
1212	88	60	26 (43.3)	34 (56.7)	25
1213	135	85	60 (70.6)	25 (29.4)	44
1216	35	22	5 (22.7)	17 (77.3)	13

[a] Plants with bacterial blight did not flower.
[b] This cross was discontinued from this study.

Table 3.3 Chi-square analysis for goodness of fit for the possible ratio determining fragrant inheritance in crosses of non-fragrant × fragrant and fragrant × fragrant *Anthurium* plants.

Cross no.	Flowered		Ratio 1:1[a]	
	F	NF	χ^2	*P*
1172	11	6	1.47	0.25-0.10
1180[b]	67	14	34.68	<0.00
1182	17	2	11.84	<0.00
1183	4	1	1.80	0.25-0.10
1184	4	5	0.20	0.75-0.50
1185	5	5	0.00	>0.95
1188	54	23	12.48	<0.00
1195	31	55	6.70	0.01-0.005
1196	27	19	1.39	0.25-0.10
1197	4	7	0.82	0.75-0.50
1198	20	5	9.00	<0.00
1200	14	7	2.33	0.25-0.10
1201	2	22	16.67	<0.00
1202	8	5	0.69	0.50-0.25
1203	27	12	5.77	0.05-0.01
1204	46	32	5.12	0.05-0.01
1205	17	18	0.03	0.90-0.75
1206	15	34	7.37	0.01-0.005
1207	24	14	2.63	0.25-0.10
1208	8	13	1.19	0.50-0.25
1210	10	19	2.79	0.05-0.01
1212	26	34	1.07	0.75-0.50
1213	60	25	14.41	<0.00
1216[c]	5	17	6.54	0.05-0.01

[a] For a ratio of 1:1, Fragrant (Ff) × Non-fragrant (ff or FF) , fragrance is produced in heterozygous (Ff) form.

[b] Both parents are fragrant. For a ratio of 3:1, F : NF, fragrance is produced in Fragrant (Ff) × Fragrant (Ff); $\chi^2 = 3.09$, $P = 0.10\text{-}0.05$

[c] Both parents are fragrant. For a ratio of 3:1, F : NF, fragrance is produced in Fragrant (Ff) × Fragrant (Ff); $\chi^2 = 32.06$, $P < 0.01$

Both parents in cross number 1180 ('Trinidad' and *A. lindenianum*) and 1216 (*A. armeniense* and UH1131) were fragrant and produced a population of fragrant and non-fragrant plants. Results from chi-square analysis, testing for the possibility that both parents were heterozygous for fragrance was insignificant. The expected ratio for this cross should have been 3 : 1, fragrant : non-fragrant (Table 3.3).

Taken together, chi-square analyses suggest that the number of genes controlling fragrance in *Anthurium* should be more than one. These results were similar to those in *Gladiolus* (McLean, 1933) and are not unexpected. At least three major compounds contribute to detectable fragrance in *Anthurium* (see Chapter 4; Kuanprasert *et al.*, submitted). Interestingly, genetics of fragrance in some popular commercial crops such as rose has not been reported. In rose, inheritance of fragrance is also believed to be complex; crosses among non-fragrant roses yielded fragrant progenies whereas those among fragrant roses yielded non-fragrant progenies (K. Zary, Jackson & Perkins, personal communication). In *Anthurium*, a hypothesis of a quantitative basis for fragrance inheritance should be tested.

Inflorescences from the same plant that flowered at different time yielded the same result for both spathe and spadix colors and type of fragrance. Several types of fragrance, including non-parental types, were found among progenies within the shared parents (Table 3.4). Parental plants *A. lindenianum*, *A. nymphaefolium* and RS1361-1 emit minty fragrance whereas *A. armeniense* and *A. hoffmanii* release a sweet floral type. Progenies from cross numbers 1185, 1195, 1200, 1202, 1203, 1204, 1210 and 1212, each with a parent that emits minty fragrance, had minty as well as other scents. It would be valuable

to examine the ratio between different type of fragrances using chemical component analysis, as done in *Ocimum basillicum* var. *glabratum* (Gupta, 1994), to determine the number of genes that control fragrance production in *Anthurium*.

Results showed that there was no linkage between the presence of flower fragrance and spathe color (Table 3.5). All the fragrant parental plants are white. Fragrant progenies possessed variation of spathe colors, coral, orange, pink or red spathe.

This study showed that fragrance is transmissable with a complex inheritance, as suggested previously (Kamemoto and Kuehnle, 1996). Breeding a commercial-quality *Anthurium* for fragrance cannot be achieved without other important horticultural characters such as flower shape and color, plant size and vase life. For this reason, genetic engineering might be suitable as a tool for adding the fragrance trait directly to an existing cultivar. One major fragrant gene, *Lis*, encoding S-linalool synthase, was isolated from *Clarkia breweri* flowers (Dudareva *et al.*, 1996). Inserting a gene that controls fragrance production into *Anthurium* using existing transformation protocols (Kuehnle and Chen, 1994) may take less time compared with the conventional method, requiring about 10 years, to obtain fragrance introgressed with other desirable characters.

Table 3.4 Spathe and spadix colors and type of fragrance from each *Anthurium* cross that flowered during 1994-1996 and the fragrant parent used.

Cross no.	Color[a]							Type of fragrance	Fragrant parent[b]
	Spathe				Spadix				
	C	P	R	W	G	R	Y		
1172	1	2	1	13	6	8	3	minty	A220-2 M
1180	-	29	-	52	19	45	17	minty, sweet	Trinidad F, A220-2 M
1181	-	-	-	7	-	6	1	minty	Trinidad F, A213-2 M
1182	-	10	8	1	6	8	5	floral, minty	A220-2 M
1183	-	-	5		-	-	5	minty	A220-2 M
1184	-	-	-	9	3	3	3	minty	A220-2 M
1185	-	10	-	-	-	10	-	minty, sweet	A220-2 M
1188	2	42	23	10	41	12	24	minty	A220-2 M
1195	-	30	31	24	60	5	20	minty, sweet, floral	RS1361-1 M
1196	-	5	17	24	10	18	18	minty	A220-2 M
1197	-	1	3	4	4	-	4	minty	A220-2 M
1198	-	3	11	11	6	2	17	floral	A220-2 M
1200	-	-	5	16	8	-	13	minty, floral, sweet	A220-2 M
1201	-	-	24	-	3	1	20	minty	A212-2 M
1202	-	-	13	-	1	-	12	floral, minty	A220-2 M
1203	2	2	11	24	8	17	14	sweet, floral	A170 M
1204	10	11	4	53	47	3	28	sweet, floral	RS1361-1 M
1205	3	-	32	-	2	13	20	minty	A220-2 M
1206	-	-	49	-	10	3	36	very light mint	A212 M
1207	-	-	38	-	20	-	18	floral	A220-2 M
1208	-	-	21	-	2	1	18	minty, light floral	A212 M
1210	-	-	29	-	16	-	13	floral, minty	A220-2 M
1212	-	-	55	-	20	-	25	fruity, floral, minty	A220-2 M
1213	-	-	-	85	1	76	8	sweet, floral	A382 M
1216	2	42	23	10	41	12	24	light floral	649-7 F

[a] C = coral; G = green; P = pink; R = red; W = white; Y = yellow.

[b] A212 and A212-2 = *A. hoffmanii* (minty); A213-2 = *A. nymphaefolium* (minty); A220-2 = *A. lindenianum* (minty); A382 = *A. armeniense* (sweet); RS1361-1 (minty) = *A. lindenianum* (A170) × *A. lindenianum* (A220); 649-7 (sweet, floral) = *A. andraeanum* 'Manoa Mist' × *A. armeniense* (A382); M = male parent, F = female parent

Table 3.5 Number of fragrant and non-fragrant progenies possessing different spathe and spadix colors for each *Anthurium* cross that flowered during 1994-1996.

Cross no.	Spathe color[a]								Spadix color[b]					
	C		P		R		W		G		R		Y	
	F	NF	F	NF	F	NF	F	NF	F	NF	F	NF	F	NF
1172	0	1	1	1	0	1	10	3	4	2	6	2	1	2
1180			22	7			44	8	10	9	43	2	11	6
1181							2	5			2	4	0	1
1182			10	0	6	2	1	0	4	2	8	8	3	2
1183					4	1							4	1
1184							4	5	0	3	3	0	1	2
1185					5	5					5	5		
1188	1	1	23	19	22	1	7	3	27	14	6	6	18	6
1195			10	20	15	16	6	18	21	39	1	4	9	11
1196			3	2	11	20	12	12	7	3	13	5	7	11
1197			1	0	2	1	4	0	3	1			4	0
1198			2	1	9	2	9	2	5	1	2	0	12	5
1200					4	1	10	6	4	4			10	3
1201					2	22			0	3	0	1	2	18
1202					8	5			1	0			7	5
1203	2	2	1	1	8	3	16	8	5	3	12	5	10	4
1204	4	6	4	7	3	1	35	18	26	21	2	1	17	11
1205	2	1			14	18			0	2	7	6	9	11
1206					15	34			4	6	0	3	11	25
1207					24	14			11	9			10	18
1208					8	13			2	0	0	1	6	12
1210					10	19			7	9			3	10
1212					21	35			7	13			14	11
1213							60	25	1	0	53	23	6	2
1216	1	0	1	0	3	16			0	2	3	3	2	11

[a] C = coral, P = pink, R = red and W = white

[b] G = green, R= red and Y = yellow.

F = fragrant flower, NF = non-fragrant flower.

References

Anderton, E. and R. Park. 1989. *Growing gladiolus*. Christopher Helm, London. 166 pp.

Bown, D. 1988. *Aroids*. Timber Press, Portland, Oregon. 256 pp.

Croat, T. B. 1980. Flowering behavior of the neotropical genus *Anthurium* (Araceae). *Amer. J. Bot.* 67: 888-904.

Dudareva, N., L. Cseke, V. M. Blance and E. Pichersky. 1996. Evolution of floral scent in *Clarkia*: novel patterns of *s*-linalool synthase gene expression in the *C. breweri* flower. *Plant Cell* 8: 1137-1148.

Gupta, S. C. 1994. Genetic analysis of some chemotypes in *Ocimum basilicum* var *glabratum*. *Plant Breed.* 112: 135-140.

Jodon, N. E. 1944. The inheritance of flower fragrance and other characters in rice. *J. Amer. Soc. Agron.* 36: 844-848.

Kamemoto, H. and A. R. Kuehnle. 1996. *Breeding anthuriums in Hawaii*. University of Hawaii Press, Honolulu. 132 pp.

Kuanprasert, N., A. R. Kuehnle and C. S. Tang. 1997. (in submission)

Kuehnle, A. R. and F. R. Chen. 1994. Genetic transformation in *Anthurium*. (*In*) Y. P. S. Bajaj, ed. *Biotechnology in agriculture and forestry*. Springer-Verlag, Berlin. pp. 217-225

McLean, F. T. 1933. The inheritance of fragrance in gladiolus species crosses. *The New York Bot. Gar.* 34: 73-80.

McLean, F. T. 1938. A genetic analysis of the inheritance of fragrance in gladiolus. *Bull. Torr. Bot. Club* 65: 181-197.

Srb, A. M., R. D. Owen and R. S. Edgar. 1965. *General genetics*. W. H. Freeman and Company, San Francisco. 557 pp.

CHAPTER 4

CHEMICAL ANALYSIS OF FRAGRANT *ANTHURIUM* SPECIES AND HYBRIDS

4.1 Abstract

Fragrant spadices of seven *Anthurium* species and ten hybrids were analyzed by gas chromatography and mass spectrometry for their chemical components. Nineteen monoterpenes were identified with somes alcohols, aldehydes and esters. Limonene and 1,8-cineole were common to most samples along with α-pinene, β-pinene, myrcene and linalool. Scented *Anthurium* plants were classifed as floral, minty, pine and minty. Hybrids of scented species showed the mixtures of sweet and floral. Qualities of hybrid UH1299 emitting a sweet and floral scent all day differed at different time of the day. The amounts and types of compounds produced varied during the daily cycle.

4.2 Introduction

Fragrance recently has been reintroduced as an objective in breeding programs of commercial crops such as rose, carnation and gladiolus (Barletta, 1995). In anthurium, fragrance is rarely found among hybrids on the market. Introducing a new feature such as fragrance is expected to enhance the marketability of the flower (Halloran and Kuehnle, in

preparation). A majority (58%) of wholesale and retail florists indicated fragrance to be a desirable character.

Only eleven species of *Anthurium* were previously described as fragrant (Bown, 1988; Croat, 1980). There is one report on scent chemical composition, that of *A. ochranthum*. Two major monoterpenes, ipsdienol (2-methyl-6-methylene-2, 7-octadien-4-ol) and myrcene were identified (Whitten *et al.*, 1988).

In addition, new compounds and new compositions of fragrant plant species are actively sought to produce new fine fragrances for the perfumery industry (Surburgh *et al.*, 1993; Tollsten *et al.*, 1994).

The present study reports the chemical composition of fragrance in 17 *Anthurium* species and hybrids. Variation in the daily cycle of scent emission in one hybrid is present. These results will aid descriptive analysis of *Anthurium* scent and our understanding of the genetics of fragrance inheritance.

4.3 Material and Methods

4.3.1 Extraction Methods

Two extraction methods were used. In solvent extraction, a scent-emitting flower was cut at the greenhouse and brought to the laboratory. The spadix was removed and soaked in methylene chloride. The amount of methylene chloride and the size of vial used varied depending on the size of the spadix. Generally, for small spadices such as from *A. amnicola* and *A. antioquiense*, 5 ml of methylene chloride in vial size 1 × 5 cm was used.

For larger spadices such as from *A. lindenianum* and *A. ochranthum*, 15-20 ml of methylene chloride in vial size 1.25 × 15 cm was used. The extract was concentrated for about 1-1.5 hours under gentle nitrogen gas stream to a final volume of 1-1.5 ml. Samples were stored at -12 C.

In vacuum headspace (Brunke *et al.*, 1992), a closed-loop stripping apparatus (courtesy from DRAGOCO, Holzminden, Germany) was used for trapping volatile compounds from scented anthuriums (Fig. 4.1). In this method, the spathe and spadix of an emitting inflorescence was inserted into a 250 ml Erlenmeyer flask, with two outlets near the bottom of the flask. One outlet was connected to an absorbent column, packed with XAD-4 resin (120 mg, Supelco, Bellefonte, Pensylvania) in line with a vacuum pump (Model DD-20, Fisher Scientific, Springfield, New Jersey). The vacuum pump was connected back to the other outlet of the flask in order to complete the air movement cycle (air recirculation). Flow rate was 150±5 ml/min. To prevent outside air from interfering with the system, paper towel was used to plug the gap between the flask neck and the peduncle. Activated charcoal (20/24 mesh) was used to purify the air that exited the vacuum pump before passing through the flask.

Volatile compounds were trapped for 3 hours. The resin was transferred and bathed in 2 ml of methylene chloride for one hour on a shaker to desorb compounds. The extract was transferred to a new vial and concentrated by gentle nitrogen gas stream to a final volume of 0.5 ml. Samples were stored at -12 C.

Figure 4.1 Closed-loop stripping apparatus, trapping volatile compounds from an *Anthurium* flower. 1 = XAD-4 resin, absorbent for *Anthurium* scent, 2 = vacuum pump and 3 = activated charcoal to purify the air stream. Direction of flow, from 1 → 2 → 3 → flower → 1.

4.3.2 Chemical analysis

Gas chromatography (GC) analysis: Samples volumes of 2 µl each were injected into a Hewlett Packard 5890 Series II gas chromatograph (courtesy of C. S. Tang, Department of Environmental Biochemistry, University of Hawaii) equipped with a 30 m × 0.25 mm internal diameter (id.) DB-5, glass capillary column (J&W Instrument, Folsome, California) in order to separate compounds. Injector and detector port temperatures were 250 C. Initial temperature was 50 C for 2 min, increased to 150 C at the rate of 2 C/min., and then to 280 C at a rate of 15 C/min. 280 C was maintained for 10 min. The compounds with greater affinity with the stationary phase were retained in the capillary column longer that the ones with low affinity. Helium was used as carrier gas at a flow rate of 17 mm/min. When the compound eluted out of the column, it was mixed with hydrogen gas to burn. A flame ionization detector was used to detect the ionized compound. Results were reported on a HP 3396 Series II Integrator.

GC-mass spectrometry (GC-MS) analysis: Sample volumes of 1 µl each were injected into Hewlett Packard 5890 Series II GC equipped with a 30 m x 0.25 mm id. DB-5, glass capillary column, connected to a Hewlett Packard 5970 mass spectrometer. Separation conditions for GC-MS analysis was the same as described for GC-analysis. After a gaseous compound exiled the GC, it entered an ionizing region, bombarded with high energy electrons. The electron energy used in MS analysis was 70eV, multiplier voltage at 1600 V. The fragment moves in a high speed through electromagnetic lens. The mass spectrum for each compound was analyzed. Mass spectrometry was set to scan mode from 50 to 300 atomic mass units (amu). A mass spectrum was then matched with

reference compounds from the databases, NBS75K and Wiley138, which contained about 75,000 and 138,000 reference compounds, respectively. An 85% match was the minimal acceptable for compound identification.

Thirty authentic standard compounds, obtained from Aldrich Chemical Co. (Milwaukee, Wisconsin), Eastman Organic Chemicals (Rochester, New York) and Supelco (Bellefonte, Pensylvania), were subjected to GC to confirm compound verification based on retention times.

4.3.3 Plant Materials

Samples from 17 scented *Anthurium* species and hybrids were obtained by vacuum headspace (HS), solvent extraction (SE) or both methods. Due to the limit availability of *Anthurium* flowers, not all species and hybrids were extracted with both extraction methods. One inflorescence each from the species *A. armeniense*, *A. fragrantissimum*, *A. lindenianum*, *A. ochranthum* and *A. roseospadix*, and from the hybrids *A.* 'Leilani', 649-2, -7 and 1213-20 were sampled by the vacuum headspace method. One spadix each from the species *A. amnicola*, *A. armeniense*, *A. lindenianum*, and *A. ochranthum*, and from the hybrids A626, RS1361-1, 633-41, 1159-2 and 1180-95 were used in solvent extraction. Three spadices per preparation were used with species *A. antioquiense* and the hybrids *A.* 'Lady Beth' and *A.* 'Leilani'. The five species and hybrids sampled by both methods were *A. armeniense*, *A. lindenianum*, *A. ochranthum*, 649-7 and *A.* 'Leilani'.

Extraction or headspace was done at the time that the flower emitted scent. All species and hybrids used in this experiment emitted scent in the morning, except

A. fragrantissimum released scent midday and *A. ochranthum* produced scent all day long.

UH1299 is known to emit scent all day and thus was chosen to determine if variation occurs in the amounts and types of compounds emitted during the daily cycle. Headspace was used to trap flower fragrance. Two spadices of UH1299 were sampled at three different times, morning (8:30 am - 11:30 pm, afternoon (1:30 - 4:30 pm) and night (7:00 - 10:00 pm).

Scents of all species and hybrids analyzed and the parentage of each hybrid are shown in Table 1. Scent was determined previously by consensus of an expert panel of 6 members. All flowers sampled were strongly scented with the exception of a medium intensity scent for *A. roseospadix* and a very weak scent for *A. antioquiense*.

Table 4.1 Types of fragrance of 17 *Anthurium* species and hybrids.

Species/hybrids (Accession no. or cross no.)	Type of fragrance
A. amnicola (A417)	minty
A. antioquiense (A490)	minty
A. armeniense (A382)	sweet
A. fragrantissimum (A662)	floral
A. lindenianum (A220-2)	minty
A. ochranthum (A670)	pine
A. roseospadix (A509)	minty
A626 (unidentified parents)	sweet, floral
A. 'Lady Beth'	sweet, floral
A. 'Leilani'	minty
A. antioquiense × 'Tatsuta Pink Obake' (UH1299)	sweet, floral
A. lindenianum (A170) × *A. lindenianum* (A220) (RS1361-1)	minty
(*A. lindenianum* × *A. amnicola*) × *A. lindenianum* (633-41)	minty
'Manoa Mist' × *A. armeniense* (649-2, -7)	sweet, floral
('Manoa Mist' × *A. armeniense*) × *A. armeniense* (1159-2)	sweet, floral
'Trinidad' × *A. lindenianum* (1180-95)	minty
'Ellison Onizuka' × *A. armeniense* (1213-20)	sweet, floral

4.4 Results

4.4.1. Species

Monoterpenes were common compounds found in all samples. Limonene was found in all species, regardless of sampling method. Other major compounds found in each species varied. In minty *A. amnicola* prepared by solvent extraction, limonene comprised 87.16% of the sample along with xylene and β-pinene (Table 4.2, Fig. 4.2). In *A. antioquiense* of light minty scent, an unknown (RT 41.728) was found as a major compound in solvent extracts along with 1,8-cineole, benzyl alcohol, benzyl acetate, α-terpineol and carvone (Table 4.2, Fig. 4.2). In floral-scented *A. fragrantissimum* prepared by headspace, linalool (93.76%) was found as a major compound (Table 4.2, Fig. 4.3). In minty *A. roseospadix* prepared by headspace, three major compounds were found: α-pinene (20.90%), β-pinene (32.86%) and 1,8-cineole (39.89%) (Table 4.2, Fig. 4.3).

Chemical components and their relative amounts differed greatly between samples prepared by the headspace or solvent extraction methods. In *A. armeniense* possessing sweet fragrance, sabinene, β-pinene, myrcene, limonene, 1,8-cineole, α-terpinolene and methyl benzoate were present in samples from both methods (Table 4.3). However, the compounds toluene, 5-hydroxycineole and unknowns (RT 19.469 and 28.253) were found only in headspace preparations, whereas xylene and γ-terpinene were found only by solvent extraction preparation. In minty *A. lindenianum*, toluene, α-pinene, sabinene, β-pinene, limonene, 1,8-cineole and unknown (RT 19.819) were found in samples of both methods but they differed quantitatively (Table 4.3). The compounds myrcene, methyl

benzoate and linalool were found only using headspace extraction. Other compounds, namely benzyl acetate, benzaldehyde, γ-terpinene, α-terpinolene, α-terpineol, *cis*-dihydrocarvone, nerol, 5-hydroxycineole, indole and unknowns (RT 19.469, 28.253, 29.375, 31.335, 32.887, 32.985, 33.657, 36.931, 40.600 and 41.280) were found only in solvent extracts. In *A. ochranthum* possessing pine fragrance, β-pinene, myrcene and 1,8-cineole were identified as major compounds in the headspace (Table 4.3). Indole and unknown (RT 20.591) were major compounds in preparations based on solvent extraction. Some other compounds found in both methods were limonene and *cis*-dihydrocarvone. Chromatograms derived from two different extraction methods of *A. armeniense*, *A. lindenianum* and *A. ochranthum* are shown in Figs. 4.4 - 4.6.

As many scent compounds have relatively high volatility, this study focussed on those compounds with GC retention times between 3 - 32 min. Compounds at later retention times were identified as waxes, decane, eicosane, heneicosane, hexadecane, nonadecane and pentacosane (Appendix 2). These wax compounds were present only in solvent extracts.

Table 4.2 Percentage of chemical components present in fragrant *Anthurium* species based on vacuum headspace analysis and solvent extraction.

Chemical component	GC retention time (min.)	% Area			
		A. amnicola	*A. antioquiense*	*A. fragrantissimum*	*A. roseopadix*
		SE	SE	HS	HS
toluene	3.555[m,s]	-	-	3.04	0.18
xylene	7.090[m]	8.19	0.72		
α-pinene	8.285[m,s]	-	-	-	20.90
β-pinene	10.177[m,s]	4.65	-	-	32.86
limonene	13.050[m,s]	87.16	trace	2.28	0.88
1,8-cineole	13.275[m,s]	-	8.14	-	39.89
benzyl alcohol	13.942[m,s]	-	7.65	0.92	0.54
γ-terpinene	14.857[m]	-	-	-	0.24
α-terpinolene	16.652[m]	-	-	-	0.35
linalool	17.556[m,s]	-	-	93.76	0.93
phenylethyl alcohol	17.592[m,s]	-	trace	-	-
unknown	19.469	-	-	-	0.22
unknown	19.819	-	-	-	1.77
benzyl acetate	21.687[m,s]	-	11.48	-	-
α-terpineol	23.419[m]	-	2.27	-	-
cis-dihydrocarvone	24.275[m]	-	trace	-	0.30
nerol	26.402[m,s]	-	trace	-	-
carvone	26.855[m,s]	-	0.21	-	-
unknown	28.253	-	-	-	0.94
unknown	41.728	-	68.53	-	-

HS = preparation using vacuum headspace method.
SE = preparation using solvent extraction.
[m] comparison of MS and relative retention time with published data.
[s] identification confirmed by GC-retention time of authentic reference compounds.
trace = amount less than 0.10%.

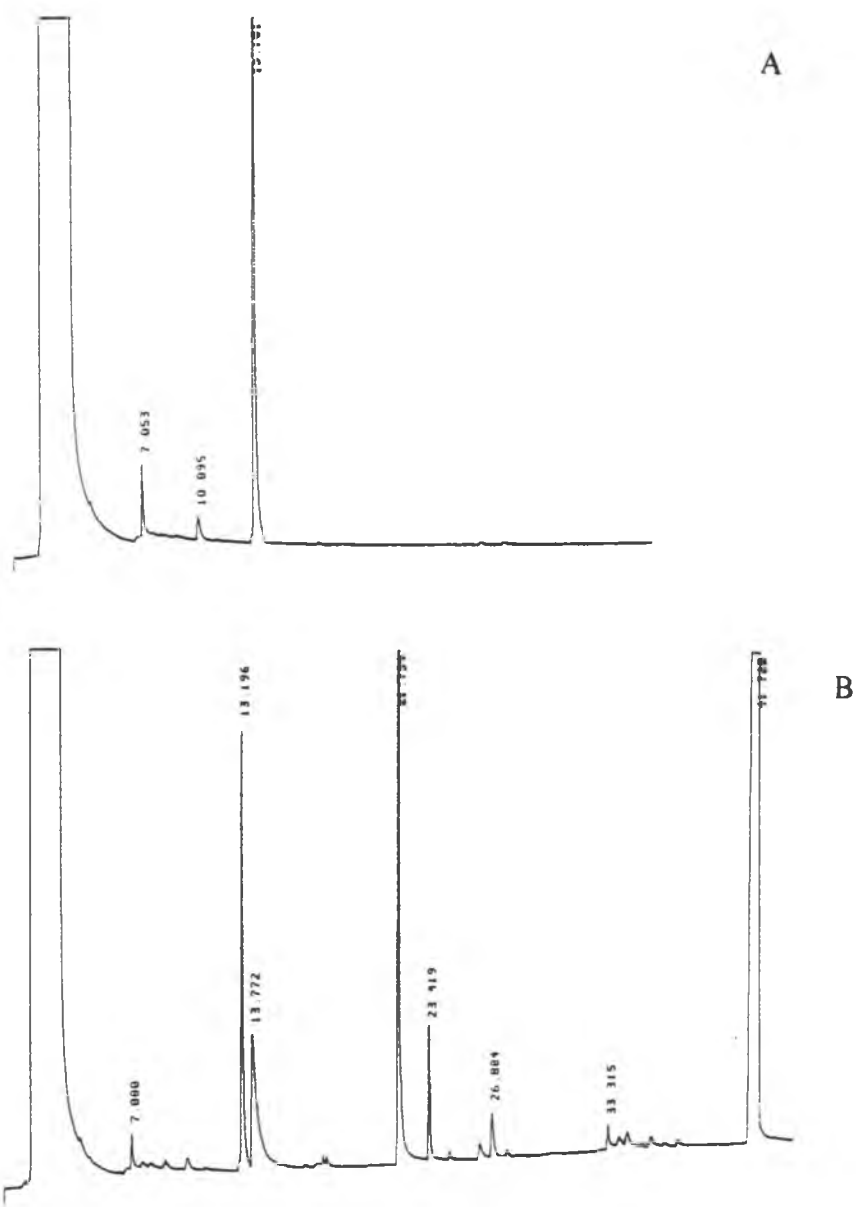

Figure 4.2 Chromatograms of *A. amnicola* (A) and *A. antioquiense* (B) using solvent extraction.

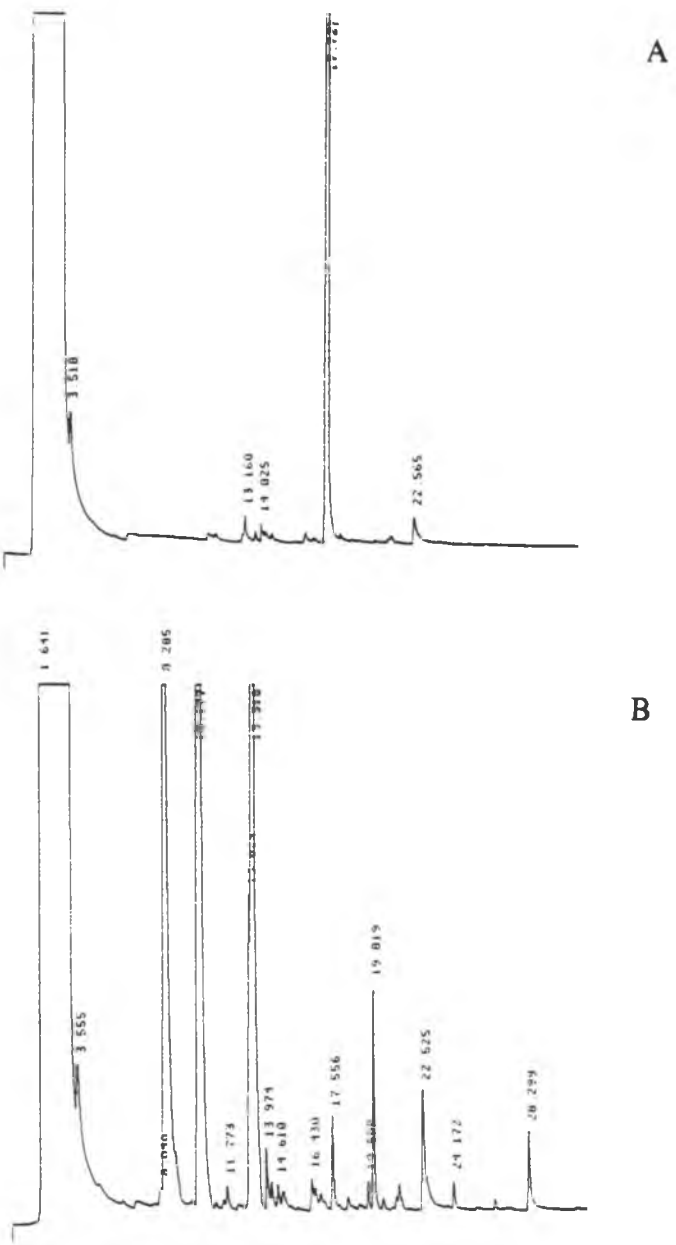

Figure 4.3 Chromatograms of *A. fragrantissimum* (A) and *A. roseospadix* (B) using headspace

Table 4.3 Comparison of percentage of chemical components present in fragrant *Anthurium* species based on samples prepared by vacuum headspace and solvent extraction.

Chemical compounds	GC -retention time (min.)	% Area					
		A. armeniense		*A. lindenianum*		*A. ochranthum*	
		HS	SE	HS	SE*	HS	SE
toluene	3.555[m,s]	trace	-	0.20	0.33	-	trace
xylene	7.090[m]	-	0.61	-	-	-	-
α-pinene	8.285[m,s]	2.49	trace	30.60	0.30	3.75	-
benzaldehyde	9.513[m,s]	-	-	-	0.12	-	-
sabinene	10.177[m,s]	13.17	7.93	18.79	0.19	-	-
β-pinene	10.177[m,s]	5.99	3.60	15.66	0.16	21.48	trace
myrcene	11.114[m,s]	2.30	2.30	1.63	-	11.66	trace
α-terpinene	12.384[m,s]	-	1.01	-	-	-	-
limonene	13.050[m,s]	0.71	1.75	trace	trace	1.25	trace
1,8-cineole	13.275[m,s]	67.54	53.69	26.18	5.05	55.83	5.06
benzyl alcohol	13.942[m,s]	-	-	-	-	-	-
γ-terpinene	14.857[m]	-	1.18	-	trace	-	-
cis-sabinene hydrate	15.380[m]	-	-	-	-	0.66	-
α-terpinolene	16.652[m]	0.85	1.54	-	0.12	0.53	-
methyl benzoate	17.125[m]	2.49	26.39	0.57	-	-	-
linalool	17.756[m,s]	-	-	0.38	-	-	1.45
phenylethyl alcohol	17.592[m,s]	0.52	trace		-		
unknown	19.469	0.21	-	-	0.93		
unknown	19.819	-	-	5.99	4.32	-	-
unknown	20.591	-	-	-	-	4.28	16.71
benzyl acetate	21.687[m,s]	-	-	-	33.88	-	-
α-terpineol	23.419[m,s]	trace	trace	-	2.91	-	3.95
cis-dihydrocarvone	24.275[m]	0.19	trace	-	2.45	0.56	trace
dihydrocarvone	25.109[m,s]	-	-	-	0.46	-	-
nerol	26.402[m,s]	-	-	-	0.12	-	-
5-hydroxy-cineole	26.934[m]	trace	-	-	1.81	-	1.58
unknown	28.253[m]	3.54	-	-	24.67	-	trace
unknown	29.375	-	-	-	0.43	-	-
indole	30.932[m,s]	-	-	-	0.15	-	71.52

HS = preparation using vacuum headspace method.
SE = preparation using solvent extraction.
[m] comparison of MS and relative retention time with published data.
[s] identification confirmed by GC-retention time of authentic reference compounds.
trace = amount less than 0.10%.
* other unknown results after RT 31.000 are shown in Table 5.

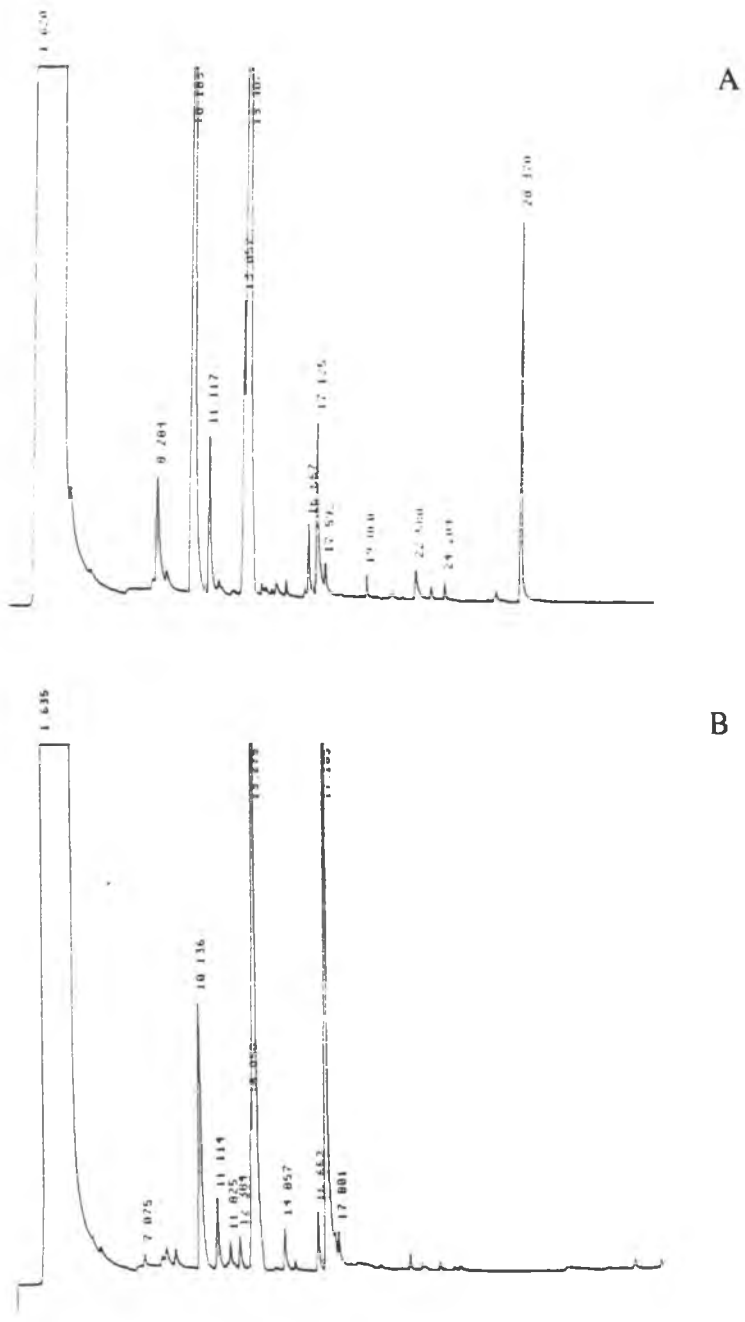

Figure 4.4 Comparison of chromatograms of *A. armeniense* derived from samples prepared by headspace (A) and solvent extraction (B).

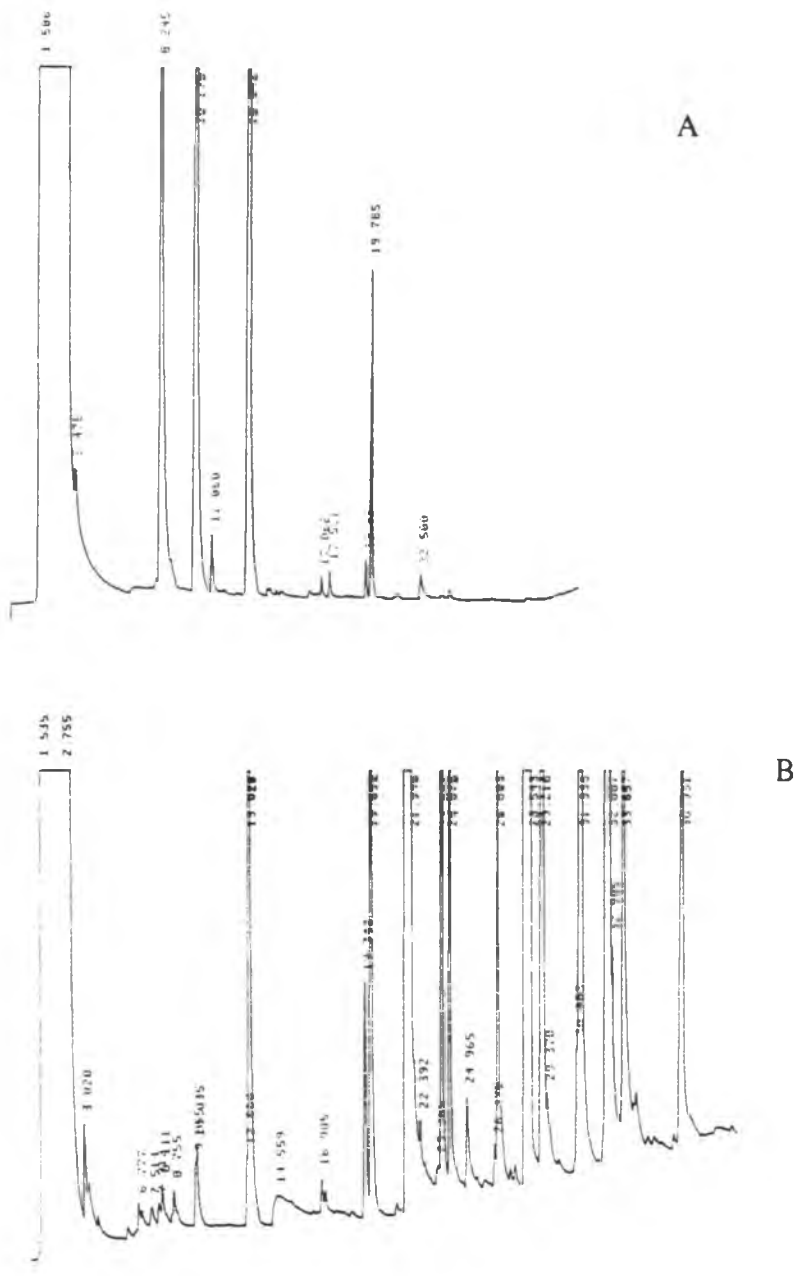

Figure 4.5 Comparison of chromatograms of *A. lindenianum* derived from samples prepared by headspace (A) and solvent extraction (B).

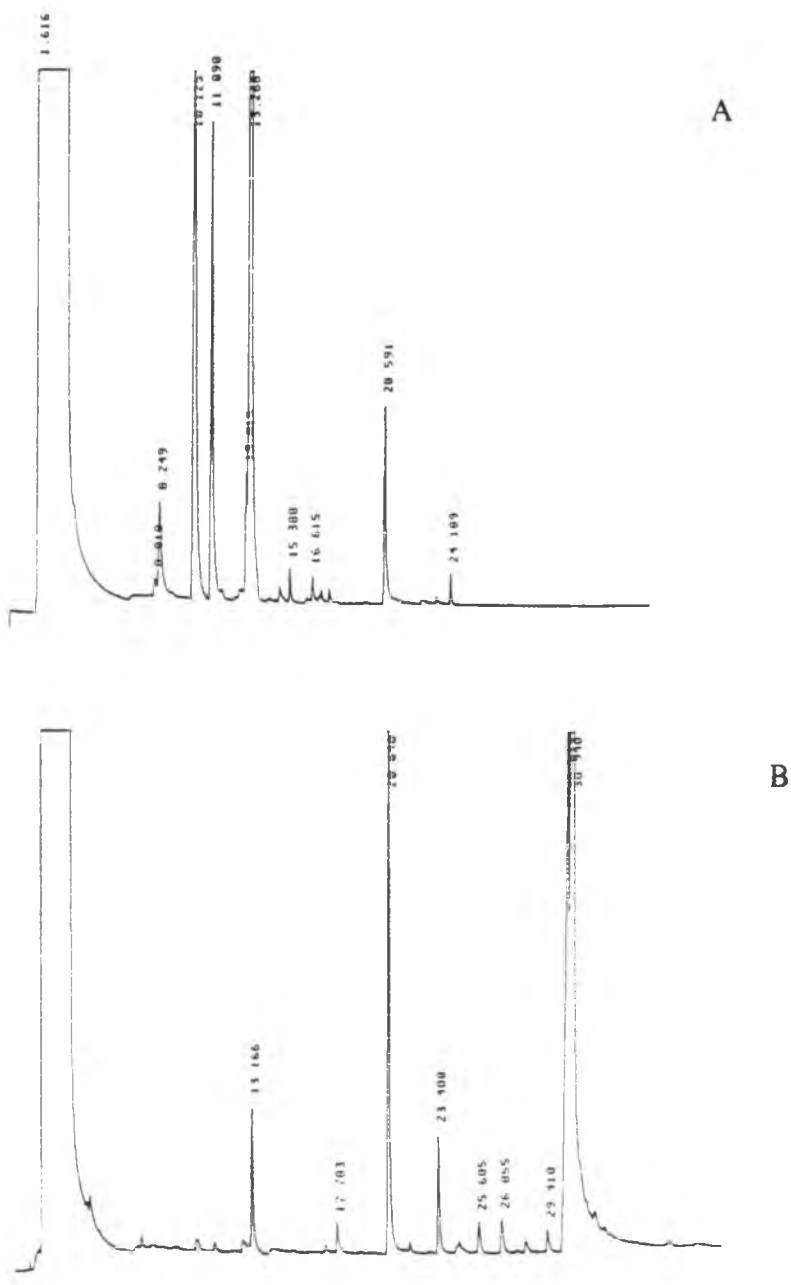

Figure 4.6 Comparison of chromatograms *A. ochranthum* derived from samples prepared by headspace (A) and solvent extraction (B).

4.4.2 Hybrids

Among the ten anthurium hybrids analyzed, seven were derived from *A. amnicola*, *A. antioquiense*, *A. armeniense*, and/or *A. lindenianum* (Table 4.1). Headspace samples obtained from the hybrids 649-2, 649-7 and 1213-20, sharing *A. armeniense* as a parent, all contained toluene, sabinene, β-pinene, myrcene, limonene, 1,8-cineole and phenylethyl alcohol but in differing amounts (Table 4.4). Solvent extraction samples from the hybrids 649-7 and 1159-2, also derived from *A. armeniense,* differed from those prepared using the headspace method. The major (more than 10%) compounds found in these two hybrids were phenylethyl alcohol and benzyl acetate. Other two major compounds, toluene and unknown (RT32.984) were found only in 649-7 whereas limonene was found in 1159-2.

Results obtained via solvent extraction from *A. lindenianum* hybrids 633-41, 1180-95 and RS1361-1 were similar to the parent *A. lindenianum.* However, relative compound amounts differed (Table 4.5). 1,8-cineole (32.00%) was found in a greater amount for 633-41 whereas only 1-8% was found in others. α- pinene and β-pinene were found in relatively low amounts (less than 1%) in all samples. The amount of α-terpineol (5.79%) was greater in RS1361-1 than in the others. 5-hydroxycineole at 7.20% was greater in 1180-95 than in the others (1.50-2.00%). A major compound found in all samples was unknown (RT 28.755), with more than 50% of this compound found in 1180-95 and RS1361-1 and about 15-25% found in *A. lindenianum* and 633-41.

The three hybrids, *A.* 'Leilani', *A.* 'Lady Beth' and A626, emitted mixtures of fragrances (Table 4.6). Results from minty and sweet *A.* 'Leilani' obtained from both headspace and solvent extractions, showed six different compounds β-pinene, 1,8-cineole, benzyl alcohol, linalool, benzyl acetate and α-terpineol, were present but differed quantitatively between methods. 1,8-cineole was found as a major compound in both methods. In solvent extracts, benzyl alcohol, benzyl acetate and α-terpineol were found in greater amounts than those in headspace. In *A.*'Lady Beth' emitting a sweet and floral scent, 1,8-cineole, linalool, benzyl acetate and unknown (RT 17.14) were major compounds. α-terpineol and 1,8-cineole were major compounds found in the sweet and floral scent of A626.

Table 4.4 Chemical component comparison of fragrant progenies from crosses 649, 1159 and 1213, derived from a parental *A. armeniense* and based on vacuum headspace or solvent extraction.

Compounds	GC retention time (min)	% Area						
		Headspace				Solvent extraction		
		A382	649-2	649-7	1213-20	A382	649-7	1159-2
toluene	3.555[m,s]	trace	3.55	2.49	6.43	-	10.41	-
1-butanol, 3-methyl-acetate	6.255[m]	-	16.53	-	-	-	-	-
xylene	7.034[m]	-	-	-	-	0.61	-	2.50
α-pinene	8.285[m,s]	2.49	-	5.47	1.62	trace	-	-
sabinene	10.177[m,s]	13.17	8.89	8.20	8.79	7.93	-	-
β-pinene	10.177[m,s]	5.99	4.04	3.71	3.85	3.60	-	-
myrcene	11.114[m,s]	2.30	trace	1.92	1.71	2.30	-	-
α-terpinene	12.384[m]	-	-	-	-	1.01	-	-
limonene	13.050[m,s]	0.71	1.46	23.96	1.22	1.75	-	22.73
1,8-cineole	13.275[m,s]	67.54	59.10	42.20	69.04	53.69	-	-
benzyl alcohol	13.942[m,s]	-	-	-	2.25	-	5.74	-
γ-terpinene	14.857[m]	-	-	-	-	1.18	-	-
α-terpinolene	16.652[m]	0.85	-	-	-	1.54		
methyl benzoate	17.185[m]	2.49	-	-	-	26.39	-	-
linalool	17.556[m,s]	-	-	-	-	-	-	2.67
phenethyl alcohol	18.588[m,s]	0.52	6.43	2.01	3.85	trace	13.98	26.47
unknown	19.469	0.21	-	-	-	-	-	-
unknown	19.819	-	-	10.10	-	-	-	-
unknown	21.203	-	-	-	1.24	-	-	-
benzyl acetate	21.754[m,s]	-	-	-	-	-	12.86	26.71
α-terpineol	23.381[m,s]	trace	-	-	-	trace	-	2.27
cis-dihydro-carvone	24.275[m]	0.19	-	-	-	trace	2.02	-
nerol	26.402[m,s]							
5-hydroxy-cineole	26.934[m]	trace						
linalyl acetate	27.924[m,s]	-	-	-	-	-	5.18	12.06
unknown	28.253[m,s]	3.54	-	-	-	-	2.30	-
unknown	29.055	-	-	-	-	-	6.26	-
undecanal	31.428[s]	-	-	-	-	-	4.76	4.59
unknown	32.984	-	-	-	-	-	29.57	-
unknown	33.693	-	-	-	-	-	6.92	-

A382 = *A. armeniense*, 649-2,-7 = 'Manoa Mist' × *A. armeniense*, 1159 = ('Manoa Mist' × *A. armeniense*) x *A. armeniense* , 1213 = 'Ellison Onizuka' × *A. armeniense*.

[m] comparison of MS and relative retention time with published data, [s] identification confirmed by GC-retention time of authentic reference compounds, trace = amount less than 0.10%.

Table 4.5 Chemical component comparison fragrant progenies from crosses 633, 1180 and RS1361, derived from a parental *A. lindenianum* and based on solvent extraction.

Compounds	GC retention time (min)	% Area			
		A. lindenianum	633-41	1180-95	RS1361-1
toluene	3.478[m,s]	0.33	-	-	-
xylene	7.073[m]	-	0.68	-	-
α-pinene	8.210[m,s]	0.30	0.67	0.40	0.27
benzaldehyde	9.513[m,s]	0.12	-	-	-
sabinene	10.177[m,s]	0.19	0.75	0.38	0.33
β-pinene	10.132[m,s]	0.16	0.63	0.31	0.28
limonene	13.042[m,s]	trace	trace	1.21	0.13
1,8-cineole	13.123[m,s]	5.05	32.00	1.21	6.44
γ-terpinene	14.559[m]	trace	-	-	-
α-terpinolene	16.652[m]	0.12	-	-	-
unknown	17.088	-	-	0.16	0.28
unknown	19.469	0.93	0.29	0.52	0.48
unknown	19.826	4.32	1.25	6.79	5.07
benzyl acetate	21.698[m,s]	33.88	-	5.17	5.45
α-terpineol	23.724[m,s]	2.91	1.04	0.21	5.79
cis-dihydrocarvone	24.160[m]	2.45	1.08	4.17	4.16
dihydrocarvone	25.109[m,s]	0.46	-	-	0.37
nerol	26.494[m,s]	0.12	2.02	-	-
5-hydroxycineole	26.925[m]	1.81	1.85	7.20	1.66
unknown	28.255	24.67	15.51	51.51	55.46
unknown	29.375	0.43	20.51	5.26	2.97
indole	30.932[m,s]	0.15	-	-	-
unknown	31.355	4.65	3.72	1.86	1.95
unknown	32.887	9.80	5.47	9.93	-
unknown	32.985	0.55	-	-	5.90
unknown	33.657	2.69	-	0.62	0.15
unknown	36.931	2.65	11.32	3.09	2.86
unknown	40.600	0.97	1.21	-	-
unknown	41.280	0.29	-	-	-

RS1361-1 = *A. lindenianum* × *A. lindenianum*, 633-41 = (*A. lindenianum* × *A. amnicola*) × *A. lindenianum*, 1180 = 'Trinidad' × *A. lindenianum*.

[m] comparison of MS and relative retention time with published data.

[s] identification confirmed by GC-retention time of authentic reference compounds.

trace = amount less than 0.10%.

Table 4.6 Chemical compounds found in hybrids *A.* 'Leilani', *A.* 'Lady Beth' and A626 using headspace method and/or solvent extraction.

Compounds	GC retention time (min)	% Area			
		Headspace	Solvent extraction		
		A. 'Leilani'	*A.* 'Leilani'	*A.* 'Lady Beth'	A626
toluene	3.523[m,s]	0.44	-	-	9.17
1-butanol, 3-methyl acetate	6.262[m]	0.89	-	-	-
xylene	7.101[m]	-	0.29	1.31	-
unknown	7.649	-	-	2.07	-
α-pinene	8.243[m,s]	2.21	-	2.33	25.64
camphene	8.894[s]	-	-	3.08	-
sabinene	10.144[m,s]	9.30	-	-	-
β-pinene	10.144[m,s]	7.74	1.60	1.15	5.96
myrcene	11.084[m,s]	2.56	-	-	5.83
limonene	13.017[m,s]	1.12	-	-	6.30
1,8-cineole	13.405[m,s]	63.35	46.31	20.91	38.24
benzyl alcohol	14.125[m,s]	0.23	25.78	2.45	2.18
unknown	14.320	0.53	-	-	
γ-terpinene	14.849[m,s]	0.28	-	-	-
α-terpinolene	16.622[m]	1.09	-	-	-
linalool	17.615[m,s]	4.23	5.10	27.74	6.68
unknown	19.838	3.50	-	-	-
benzyl acetate	21.810[m,s]	0.22	7.50	16.72	-
α-terpineol	23.403[m,s]	0.73	11.79	5.10	-
trans-dihydro-carvone	23.708[m]	0.50	-	-	-
dihydrocarvone	24.155[m,s]	0.49	-	-	-
exo-2-hydroxy-cineole	24.500[m]	-	0.64	-	-
unknown	28.314	0.42		-	-
unknown	41.303	-	0.99	17.14	

[m] comparison of MS and relative retention time with published data.
[s] identification confirmed by GC-retention time of authentic reference compounds.
trace = amount less than 0.10%.

4.4.3 Time of the Day

Major compounds emitted by sweet and floral UH1299 varied with time of the day as follows: morning - 1,8-cineole, undecane and benzyl acetate; afternoon - limonene, 1,8-cineole and benzyl acetate; and night - toluene, undecane and benzyl acetate. (Table 4.7, Fig. 4.7). Certain compounds were present only at a particular time of the day, such as trace amounts of benzaldehyde in the morning, myrcene in the afternoon and *cis*-dihydrocarvone and trace amounts of carvone at night. Other compounds present at all times but differing in relative amounts were decane, benzyl alcohol, phenylethyl alcohol, α-terpineol, *trans*-dihydrocarvone, nerol and unknowns (RT 18.916, 19.785, 20.258, 21.177, 28.253).

Table 4.7 Comparison of emitted fragrance of UH1299 using headspace analysis at different times of day.

GC retention time (min)	Compounds	% Area morning	afternoon	night
3.483[m,s]	toluene	3.71	9.52	20.68
6.255[m]	1-butanol, 3-methyl acetate	1.85	0.90	-
8.225[m,s]	α-pinene	5.86	7.20	-
9.123[m,s]	benzaldehyde	trace	-	-
10.162[m,s]	sabinene	5.14	6.41	-
10.162[m,s]	β-pinene	3.42	4.28	-
11.061[m,s]	myrcene	-	0.90	-
11.524[m]	decane	1.60	0.77	1.57
13.005[m,s]	limonene	9.19	11.96	-
13.148[m,s]	1,8-cineole	12.49	16.04	2.95
13.942[m,s]	benzyl alcohol	5.54	3.10	6.57
17.536[m]	undecane	12.56	5.76	11.78
18.320[m,s]	phenylethyl alcohol	2.27	1.07	2.24
18.916	unknown	1.39	0.60	1.34
19.785	unknown	7.05	4.26	4.48
20.258	unknown	1.45	0.65	1.42
21.177	unknown	4.72	2.12	4.49
21.659[m,s]	benzyl acetate	15.87	21.66	33.32
23.402[m,s]	α-terpineol	trace	trace	trace
24.122[m]	*trans*-dihydrocarvone	1.27	0.64	1.53
25.625[m]	*cis*-dihydrocarvone	-	-	1.05
26.389[m,s]	nerol	1.73	0.71	1.51
26.800[m,s]	carvone	-	-	trace
28.253	unknown	2.98	1.45	5.07

[m] comparison of MS and relative retention time with published data.
[s] identification confirmed by GC-retention time of authentic reference compounds.
trace = amount less than 0.10%.

Figure 4.7 Comparison of UH1299 chromatograms of samples obtained via headspace during morning (A), afternoon (B) and night (C). Peak at RT 22.477 is identified as naphthalene, a contaminant from the adsorbent.

A

B

C

4.5 Discussion

Five fragrance types represented by the 17 scented species and hybrids were floral, minty, pine, sweet and sweet floral. The most common fragrance compound produced by the plants were α-pinene, limonene, 1,8-cineole and linalool. These four compounds are present in more than 40 scented plant genera (Knudsen *et al.*, 1993). Nevertheless, each fragrance category had a unique chemical profile. Based on their relative amounts and the specific sensory property of the individual authentic standards, the following combinations of compounds is proposed to comprise a species scent: *A. amnicola* (minty) - xylene, β-pinene and limonene; *A. antioquiense* (light minty) 1,8-cineole, benzyl alcohol, benzyl acetate, and unknown (RT41.728); *A. armeniense* (sweet) - sabiene, β-pinene and 1,8-cineole; *A. fragrantissimum* (floral) - linalool; *A. lindenianum* (minty) - α-pinene, sabinene, β-pinene, and limonene; *A. ochranthum* (pine) - β-pinene, myrcene and 1,8-cineole; and *A. roseospadix* - α-pinene, β-pinene and 1,8-cineole.

Major compounds found in flower samples may not directly contribute to emitted scent. Myrcene, with its sweet and pleasant scent, might be the major contributor towards the sweet fragrance of *A. armeniense* although sabinene (13.17%) and 1,8-cineole (67.54%) were found as major compounds. In *Cymbidium virescens* 'Songmei' sample, nerolidol with a woody floral and green character, was found as the major (60%) component whereas methyl jasmonate and methyl epijasmonate were present at 4.3% and 1.1%, respectively (Omata *et al.*, 1990). Fragrance of 'Songmei' flower was described as floral with notes of jasmine and fresh citrus. It meant that

methyl jasmonate and methyl epijasmonate represented an unique fragrance of 'Songmei'. Similar results were found in the white ginger, *Hedychium coronarium*. Even though lactones were present in small amounts, less than 0.2%, these group of compounds contributes to the sweet note of ginger flowers (Omata *et al.*, 1991).

Extraction method played a crucial role in determining which compounds were detected in the flowers, as previously noted by Tollsten *et al.* (1994). For example, ipsdienol was reported to be a major component and myrcene a moderate component of *A. ochranthum* scent based on headspace analysis (Whitten *et al.*, 1988). In our experiment, ipsdienol was absent in both types of preparations while 1,8-cineole and β-pinene were present in 56% and 21% of the headspace samples, respectively. Myrcene comprised of 12% of the headspace. In solvent extracts, 1,8-cineole, linalool, α-terpineol, carvone, indole and an unknown (RT 20.591) were found. Differences in the presence and/or absence of certain compounds attributed to extraction method were also found for *A.* 'Leilani' and 649-7. Knudsen *et al.* (1993) suggested that headspace was more appropriate than solvent extraction for trapping flower fragrance in order to simulate emitted fragrance. On the other hand, when total organic compounds from a flower were of interest, solvent extraction was more appropriate. In this experiment, samples obtained via headspace collectively yielded a greater percentage of low molecular weight (highly volatile) compounds.

In terms of chemistry, it was difficult to control the concentration of sample employed in the experiment. Concentration varied according to the initial volume of the samples. This was especially true in solvent extraction because the initial volume solvent

used depended on the size of the spadix. Even though in headspace preparation the initial volume of solvent for resin elution was the same, it was quite difficult to concentrate the eluate down to exact same amount in all samples. However, use of chemical analysis from both preparation methods would better represent the chemical profiles of scent production than that of either method alone.

Results of headspace analysis comparison between *A. armeniense* and its progenies and that of solvent extraction comparison between *A. lindenianum* and its progenies showed the possibility that chemical profile might be used as a tool leading to an understanding of fragrant genetic inheritance in *Anthurium*. In order to obtain a comparable chemical profile in plants, the same extraction method must be used for all. Moreover, if more than one preparation could be done, the result would be more accurate.

In addition to the similarity of their morphology, leaf shape and flowering behavior, chemical profiles of two hybrids, *A.* 'Leilani' and *A.* 'Lady Beth', looked very similar. These two hybrids might share one parent. Based on their morphology and chemical profiles obtained by solvent extraction, we speculated that *A. antioquiense* might be one parent of these two hybrids. After checking with the breeder who made *A.* 'Lady Beth' hybrid, *A. antioquiense* was indeed one of the parents (Jim Georgusis, Homestead, Florida, personal communication). In another comparison, chemical profile of A626 looked similar to that of *A. armeniense*. It is possible that chemical profiles could be a useful tool to trace parents of an unknown fragrant hybrid and to study genetic inheritance in fragrant *Anthurium*. In *Coryanthes*, chemical profiles were used to classify species into different taxonomic sections (Gerlach and Schill, 1989). In

addition, uniform fragrance proflies were found in the different clones of *Coryanthes* spp.

UH1299 was one anthurium that released scent all day, being particularly strong at night. The quality of the sweet and floral scents varied subtly. The amounts and types of chemical components emitted varied during the day. This phenomenon is reported in other fragrant flowers. Endogenous circadian rhythm of fragrance emission was tested in *Hoya carnosa* (Alternburger and Matile, 1988). It was found that scent production in this plant was synchronized with light/dark changes. Mookherjee *et al.* (1990) reported that amounts of chemical components of tuberose, *Polyanthes tuberosa*, and stephanotis, *Stephanotis floribunda*, differed during the day and night samples. In tuberose, limonene was found in a greater amount during the night whereas methyl salicylate and α-terpineol were more abundant in the morning sample. In stephanotis, more *n*-hexanol was found in the morning sample whereas more methyl benzoate and 2-phenyl nitroethane were found in the night sample. This result was confirmed by Altenburger and Matile (1990). The report mentioned exact times for maximum emissions of compounds. The greatest amount of methyl benzoate and 2-phenyl nitroethane were produced around midnight; linalool was found greatest at about 6 am. However, other factors that might affect scent production were not examined. Knowledge of such factors effect would be very helpful for a breeding program.

A. amnicola, *A. antioquiense*, *A. armeniense* and *A. lindenianum* have been used frequently as parental plants for existing cultivars. Some desirable characters of these species are unique color (purple) from *A. amnicola*, and tolerance to bacterial blight from

A. antioquiense. *A. lindenianum* was one of the parents used in producing present hybrids (Kamemoto and Kuehnle, 1996). This study showed that a minty type of scent is derived from *A. amnicola* and *A. lindenianum* whereas a floral and sweet type of scent derived from *A. antioquiense* and *A. armeniense*. Their combinations of horticultural traits and genetically transmissable scent make these four species valuable for use in breeding for fragrance.

Another aspect of fragrance is the relationship between type of scent and pollinator. Since *Anthurium* species are largely protogynous (Dahlgren *et al.*, 1985), they are naturely out-crossing. Since the pollen is not wind-borne, pollinators are needed. Croat (1980) observed that several species of *Anthurium* emitted both pleasant and unpleasant odors, however, pollinators could not be seen. Only in *A. ochranthum*, bees were reported as pollinators. Subsequently, ipsdienol and myrcene were identified as compounds that attract the euglossine bee (Whitten *et al.*, 1988). In the same year, Bown (1988) mentioned that pollination of sweet-scented A. *armeniense* was associated with a bee. Our work shows myrcene to be one chemical component in *A. armeniense* scent. The association between pollinators and fragrance in *Anthurium* needs further study.

In summary, monoterpenes limonene, 1,8-cineole, α-pinene, β-pinene and linalool, were common components of *Anthurium* fragrance. Aldehydes and esters were present less frequently. Variation of scent quality during the daily cycle was due to types and amounts of compound produced during the emission period. Extraction method greatly affected chemical profiles. Combining the results of the headspace and solvent extracts yielded more complete chemical profiles. Each *Anthurium* produced a unique chemical

profile, even if classified in the same scent category. This information might be useful for species identification, chemotaxonomy and future genetic study. In addition, this study generated data that may be useful for ecological study.

References

Altenburger, R. and P. Matile. 1988. Circadian rhythmicity of fragrance emission in flowers of *Hoya carnosa* R. Br. *Planta* 174:248-252.

Altenburger, R. and P. Matile. 1990. Further observation on rhythmic emission of fragrance in flowers. *Planta* 180:194-197.

Barletta, A. 1995. Scent makes a come back. *FloraCul. Inter.* pp. 23-25.

Bown, D. 1988. *Aroids*. Timber Press, Oregon. 256 pp.

Brunke, E. J., F. J. Hammerschmidt and G. Schmaus. 1992. The headspace analysis of flower fragrances. *Dragoco Rep.* p. 3-31.

Croat, T. B. 1980. Flowering behavior of the neotropical genus *Anthurium* (Araceae). *Amer. J. Bot.* 67: 888-904.

Dahlgren, R. M. T., H. T. Clifford and P. F. Yeo. 1985. *The families of the monocotyledons structure, evolution and taxonomy*. Springer-Verlag, New York. 354 pp.

Gerlach, G. and R. Schill. 1989. Fragrance analyses, an aid to taxonomic relationships of the genus *Coryanthes* (Orchidaceae). *Plant Syst. Evol.* 168:159-165.

Kamemoto, H. and A. R. Kuehnle. 1996. *Breeding anthurium in Hawaii*. University of Hawaii Press, Honolulu, Hawaii. 132 pp.

Knudsen, J. T., L. Tollsten and L. G. Bergstrom. 1993. Floral scents- a checklist of volatile compounds isolated by head-space techniques. *Phytochem.* 33: 253-280.

Mookherjee, B. D., R. W. Trenkle and R. A. Wilson. 1990. The chemistry of flowers, fruits and spices: live vs. dead a new dimension in fragrance research. *Pure & Appl. Chem.* 62: 1357-1364.

Omata, A., S. Kakamura, K. Yomogida, K. Moriai, Y. Ichikawa and I. Watanabe. 1990. Volatile components of TO-YO-RAN flowers (*Cymbidium faberi* and *Cymbidium virescens*). *Agri. Biol. Chem.* 54: 1029-1033.

Omata, A., K. Yomogida, Y. Teshima and S. Nakamura. 1991. Volatile components of ginger flowers (*Hedychium coronarium* Koenig). *Fla. Frag. J.* 6: 217-220.

Surburgh, H., M. Guentert and H. Harder. 1993. Volatile compounds from flower: analytical and olfactory aspects. (*In*) R. Teranishi, R. G. Buttery, and H. Sugisawa, eds. *Bioactive volatile compounds from plants*. American Chemical Society, Washington DC. pp.168-186.

Tollsten, L., J. T. Knudsen and L. G. Bergstrom. 1994. Floral scent in generalistic *Angelica* (Apiaceae)- an adaptive character? *Biochem. Syst. Ecol.* 22: 161-169.

Whitten, W. M., H. G. Hills and N. H. Williams. 1988. Occurence of ipsdienol in floral fragrances. *Phytochem.* 27: 2759-2760.

CHAPTER 5

COMPARATIVE CHEMICAL AND HISTOLOGICAL STUDIES OF FRAGRANT AND NON-FRAGRANT *ANTHURIUM* SPADICES

5.1 Abstract

Chemical analysis of fragrant and non-fragrant spadices showed that volatile compounds were present only in fragrant spadices. No heat production was detected during the time of scent emission. The spadix was divided into three different zones according to flower development. The greatest amount of storage compounds was found in the middle zone, representing the receptive stage. Tepals and pistils of fragrant and non-fragrant spadices were chemically and histologically compared. Results showed that tepals were at least a primary site involved in scent production in *Anthurium*. Lipid and starch were stored in tepal and pistil cells, but no difference in their amounts was found in pistil cells of fragrant and non-fragrant samples. In tepals, the amount of lipids was significantly greater in fragrant than non-fragrant plants, whereas the amount of starch was significantly greater in non-fragrant than fragrant samples. These data support the hypothesis that high levels of lipids are associated with scent production.

5.2 Introduction

Anthurium belongs to the Araceae comprising about 1100 species (Croat, 1992). Eleven *Anthurium* species were previously identified as fragrant (Bown, 1988; Croat, 1980) with 52 more species identified as fragrant in this dissertation (Chapter 2). The spadix is the source of scent (Croat, 1980), and the general morphology of the *Anthurium* flower has been described (Higaki *et al.* 1984). However, there is no information available on structures or specialized cells associated with scent production in this genus.

Inflorescences of 13 scented aroid species outside *Anthurium* have been studied histologically. Three genera that have been studied in some detail and in which heat and scent production occurred simultaneously are *Arum*, *Sauromatum* and *Symplocarpus* (Bermadinger-Stabentheiner and Stabentheiner, 1995; Kite, 1995; Knutson, 1974; Skubatz *et al.*, 1993; Vogel, 1990). Starch was found as a storage compound for *Arum italicum* and *A. maculatum* (Vogel, 1990) and for skunk cabbage, *Symplocarpus foetidus* (Knutson, 1974). Fatty acids were stored before scent emission in voodoo lily, *Sauromatum guttatum* (*Skubatz et al.*, 1995).

In this experiment, gas chromatography (GC) and histology were used to find the structure(s) associated with scent production in *Anthurium*. Histochemical comparison determined whether amounts of storage compound differed in fragrant and non-fragrant *Anthurium* genotypes. I hypothesized that either lipid or starch was involved in scent production by being stored in cells of fragrant spadices but not non-fragrant spadices. This is the first such study in the genus *Anthurium*.

5.3 Material and Methods

5.3.1 Gas chromatography and spadix temperature

To verify the presence of scent compounds in fragrant and non-fragrant *Anthurium* hybrids, spadix of each UH1299 (fragrant) and UH1269 (non-fragrant) was cut and soaked in methylene chloride as described in Chapter 4. Gas chromatography (GC) used was Hewlett Packard 5890 Series II equipped with a 30 m × 0.25 mm internal diameter (id) DB-5, glass capillary column (J&W Instrument, Folsome, California) in order to separate compounds. Injector and detector temperatures were 250 C. A flame ionization detector was used. Conditions of GC were an initial temperature of 90 C for 1 min, increased at the rate of 8 C/min up to 280 C, and held at 280 C for 20 min. Individual flowers excised from a spadix of fragrant hybrid, UH1299, were then separated into component parts to further determine which floral structures produced scent compounds. Thirty each of pistils, tepals or stamens were placed in a 1 × 4 cm vial containing 1 ml of methylene chloride for 15 min. Extracts were concentrated by a gentle nitrogen gas stream to reduce volumes to 0.5 ml. Concentrated extracts of 2 µl were then injected into a GC for chemical analysis as described above.

To verify the fragrant and non-fragrant phenotypes, as determined by sniffing, of flowers used in histology, spadices from other inflorescences of the same plants were chemically analyzed by GC for the presence of volatile compounds prior to histological analysis. Each spadix was soaked in 5-10 ml of methylene chloride for 15 min. The extract

was concentrated by a gentle nitrogen gas stream to reduce the volume to 1-1.5 ml. Samples were analyzed as described above.

Temperature of five spadices of each fragrant and non-fragrant *Anthurium* was detected by using microscanner D501 (Exergen, Newton, Massachusetts). Tip of probe was placed about 10 cm away from the spadix. Temperature measurement was taken on the non- fragrant spadices at pistillate stage or on the fragrant spadices at the time of scent emission.

5.3.2 Histochemistry

Spadices were harvested at the pistillate stage, with two-thirds of the spadix being receptive. Because of variable maturation of flowers on an *Anthurium* spadix progressing from the base towards the tip (Croat, 1980; Higaki *et al.*, 1984), each spadix was divided into three zones designated as base, middle and tip (Fig. 5.1). Each zone was cut at about 0.7 cm long for excision of pistils and tepals. The tip contained pre-pistillate flowers. The middle zone contained mature, receptive flowers, and the base contained a mixture of receptive and post-receptive flowers.

Fragrant spadices were obtained from three genotypes: *Anthurium antioquiense*, hybrid A491 (*A. antioquiense* × *A. amnicola*), and hybrid 729 [(*A. antioquiense* × *A. amnicola*) × UH757]. Non-fragrant spadices were obtained from three hybrids: A494 (*A. andraeanum* × *A. antioquiense*), UH1554 [UH798 × (*A. antioquiense* × 'Marian Seefurth')] and UH1750 ('Momoyama' × UH1121). One spadix was sampled per genotype, three zones per spadix.

Since lipids were implicated in scent production in other aroids, *Anthurium* spadices were exposed to osmium tetraoxide vapors (2% OsO_4, 0.05 M sodium cacodylate, pH 7.0) for 15 min. in sealed petri plates in order to preserve lipids in cells before fixation. At least 20 tepals and 20 pistils (without ovules, due to mucilage contamination; see Matsumoto *et al.*, 1995) were excised from each of the three spadix zones and kept in vials, containing fixative, at room temperature for 2 hours and then at 4 C for overnight. Fixative contained 2% acroline, 1% cetylpyridinium chloride (CPC) and 4% glutaraldehyde in 0.05 M sodium cacodylate (Na cacodylate) buffer, pH 7.0 (Matsumoto *et al.*, 1995). Following fixation, specimens were washed three times with 0.05 M Na cacodylate buffer. Specimens were post-fixed in 1% osmium tetroxide in buffer for one hour and then washed three times with buffer. Dehydration was followed a standard alcohol (EtOH) series from 10% to 70%, with a 10% concentration increase every 20 min. Specimens were left overnight in 70% EtOH, at 4 C. The next day, they were brought to room temperature and dehydrated to 100% EtOH with a 10% increase every 30 min, with two subsequent transfers to 100% EtOH, specimens were infiltrated with eponate resin (Ted Pella Inc., Redding California). The proportion of EtOH and resin was adjusted over 4 days to 100% resin, beginning with a ratio of 3:1, following by 1:1 and 1:3 of EtOH: resin. This was followed by two transfers to pure resin every 24 hours. Infiltrated specimens were transferred from vials to aluminum dishes containing fresh resin and left at room temperature overnight. Then they were placed in an oven at 40 C overnight, then brought to 60 C for polymerization overnight. Once the medium had

hardened, blocks containing individual tepals and pistils were glued onto plastic rods for further processing. Three tepals and three pistils of each zone were used in evaluation.

Glass knives were cut with an LKB Knifemaker (LKB Instruments Inc., Rockville, Maryland). Specimens were sectioned with a Sorval Porter-Blum MT2-B Ultra-Microtome (Kan Sorval, Norwalk, Connecticut) at 2 μm. Sections were floated on distilled water and heated on a slide warmer at 40 C. Slides were stained with potassium iodide (IKI) or periodic acid schiff (PAS) for starch (Feder and O'Brien, 1968). Even though osmium stained the lipids sufficiently for quantification, Sudan Black B (Bronner, 1975) was employed to verify the preserved lipids.

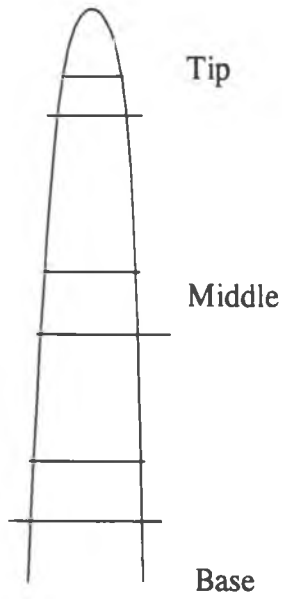

Figure 5.1 Three zones of the *Anthurium* spadix: base; middle and tip. Individual flowers of each zone were excised for histological study.

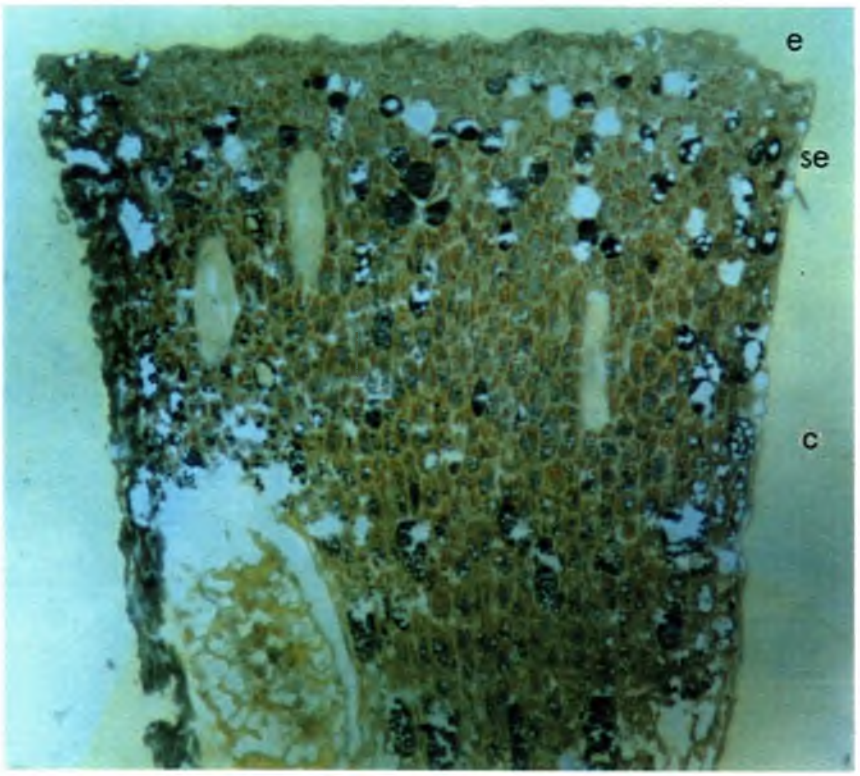

Figure 5.2 Longitudinal section of a tepal showing lipid (L) and starch (S) accumulation. e = epidermis, se = sub-epidermal and c = central (× 425).

5.3.3 Statistical Evaluation

A rating scale was used to evaluate the relative abundance of lipid and starch. Cells with the greatest amount of lipid or starch were rated as 8. Those with no detectable of lipid or starch were rated as 0. Intermediate ratings were determined by further comparisons until a graded series was obtained from 0-8. A reference set of prints was assembled for lipid or starch as shown in Fig. 5.4.

Three tepals per spadix zone per genotype were sectioned. Color pictures (× 685) of the epidermis, sub-epidermal and central areas were taken (Fig. 5.3). Three cells from each area of each tepal were arbitrarily selected for evaluation. Data per phenotype were then pooled to permit amount of lipid or starch in cells of fragrant or non-fragrant tepals in the same area of the same zone to be compared. For example, the average ratings of lipid in a total of 27 cells from the central area of the basal spadix zone of fragrant tepals were compared with the average rating in 27 cells of the non-fragrant tepals. The comparative data for fragrant and non-fragrant samples were analyzed for statistical significance by the Mann-Whitney test at the 0.05 and 0.01 probability levels (Sprent, 1993).

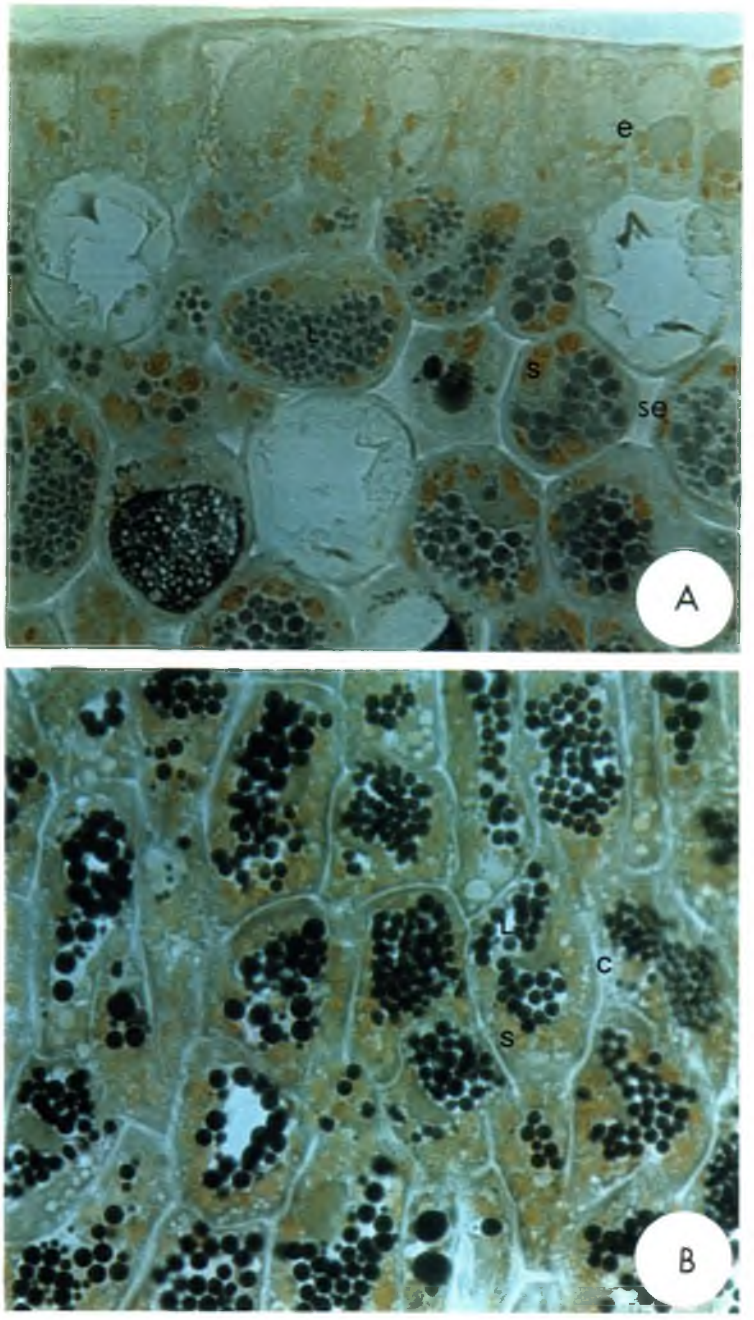

Figure 5.3 Samples of pictures used in lipid and starch evaluation in tepal cells showing (A) the epidermis and sub-epidermal area and (B) central area. e= epidermis, se = sub-epidermal, c = central area, L = lipid and S = starch (× 685).

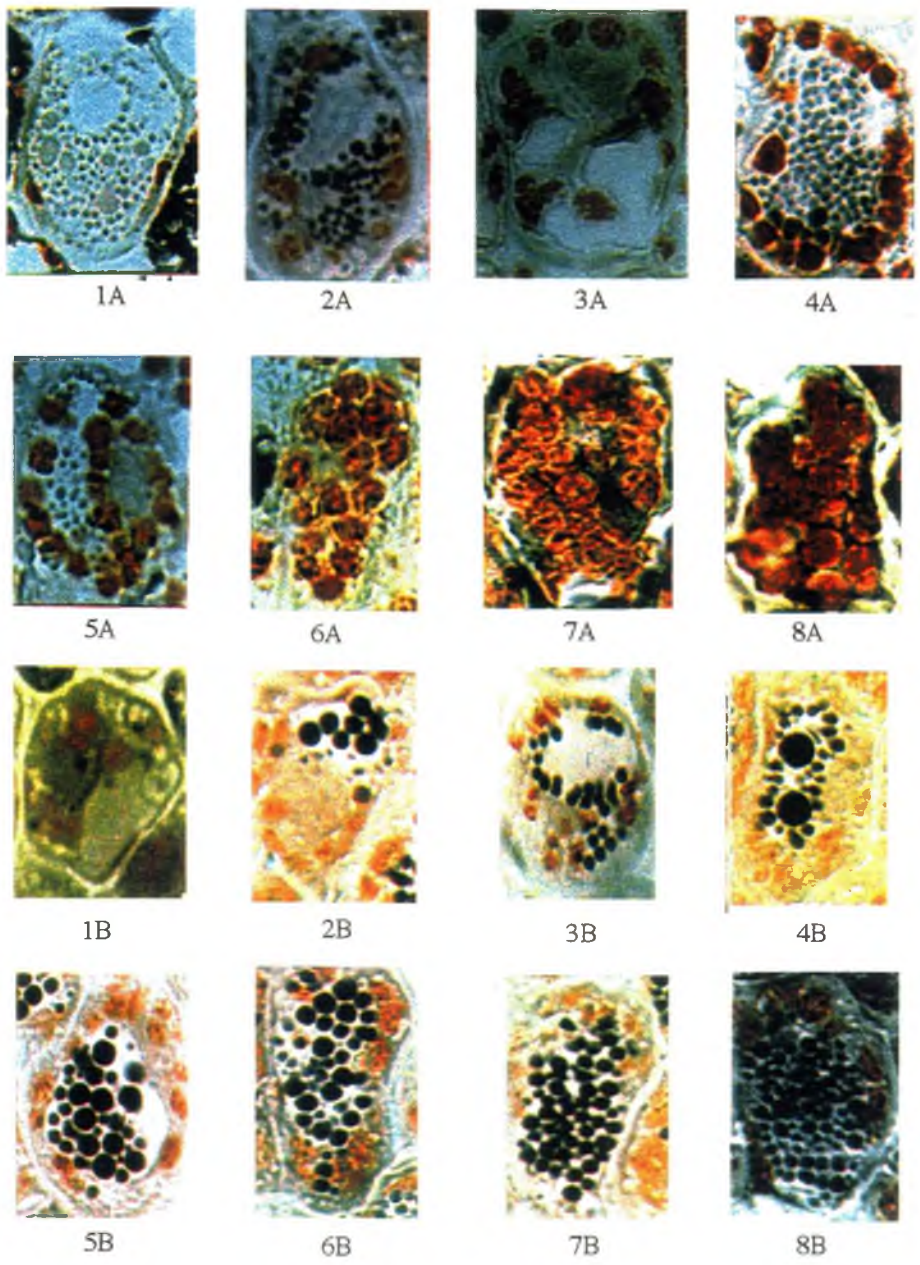

Figure 5.4 A reference set of prints assembled for evaluating the amounts of starch (A) or lipids (B) in three zones of *Anthurium* tepals. A rating of 1 indicates trace amounts and a rating of 8 represents maximal amounts.

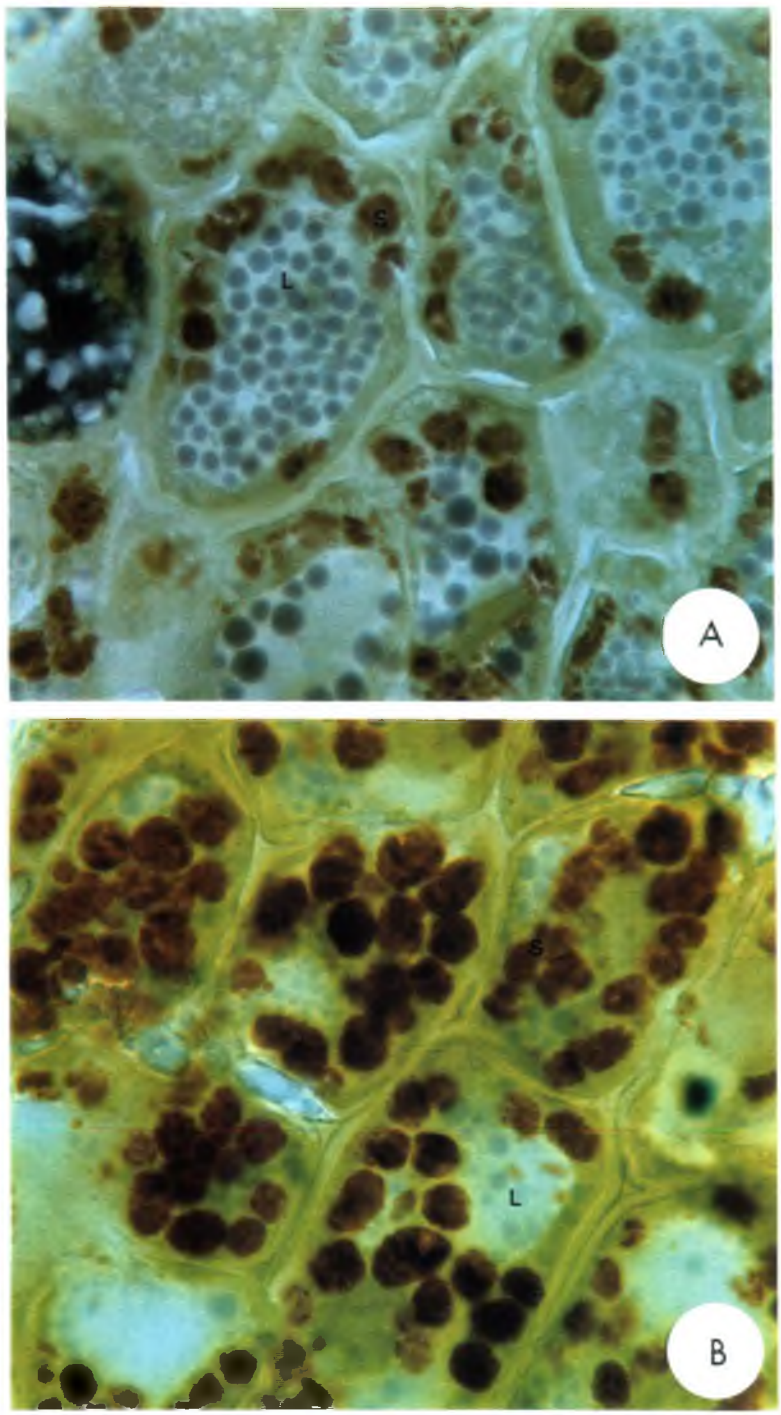

Figure 5.5 Typical cells containing lipid (A) and starch (B) in tepal cells of an *Anthurium* spadix. L = lipids and S = starch (× 4250).

5.4 Results

5.4.1 Gas chromatography and spadix temperature

Peaks at early retention times were found in extracts from the fragrant, UH1299 but not the non-fragrant, UH1269 spadices (Fig. 5.6). Those peaks were identified as fragrance compounds, mostly monoterpenes (see Chapter 4). Peaks at latter retention times, present in both fragrant and non-fragrant spadices were identified as waxes. Similar peaks at early retention times were also found in excised pistils and tepals but not stamens, of fragrant UH1299 (Fig. 5.7).

Temperature of fragrant and non-fragrant spadices was measured. The average temperature of both spadices was 26 $\pm$ 0.2 C, air temperature was 26 C. No heat production was detected from *Anthurium* spadix during scent emission.

5.4.2 Histochemistry and evaluation

Osmium vapor reacted with double bonds in lipids such as terpenes and waxes (Baker, 1958). When whole spadices of fragrant and non-fragrant were exposed to the vapor, surface tissue surrounding the stigma and the edge of tepals turned from yellow to gray or black. However, the darkening of surface tissue on the fragrant spadices was greater than that of non-fragrant spadices. After post-fixation with 1% osmium, osmophilic lipids inside cell also turned dark (Fig. 5.5). Sudan Black B did not significantly enhance lipid staining and it dislodged sections off the slide.

Potassium iodide staining was superior to periodic acid schiff for localization of starch. When polarized illumination was used to verify staining results for starch, IKI also gave the best correspondence for birefringence. Furthermore, several distinct starch grains were seen in amyloplasts stained with IKI but not PAS (Figs. 5.2 and 5.5).

Lipid and starch accumulations were found in all specimens (Appendix 4). Very little lipid was noted, and virtually no starch was present in pistils. Thus, only tepal cells were rated and data statistically evaluated.

The amount of tepal starch and lipid varied significantly between fragrant and non-fragrant samples. The Mann-Whitney test showed that the amount of lipids was significantly greater in the fragrant samples, except in the central area of the basal spadix zone (Fig. 5.8). However, there was still more lipid present in the fragrant than in the non-fragrant tepals in this region. Furthermore, the magnitude of the difference was 26%. This was similar to the degree of difference between fragrant and non-fragrant specimens in the sub-epidermal area of the basal zone, which was statistically different. The amount of lipids in the epidermis was less than that of other tissues within the same zone of fragrant and non-fragrant samples. The greatest levels of lipid accumulation in the tepals occurred in middle zone of the spadix. The range of values for all tissues and zones are showed in Appendix 4 (Table 5.4).

The opposite trend was observed for starch. The amount of starch in non-fragrant samples was more than that of fragrant samples (Fig. 5.9). Starch content was significantly greater in all cases, except for in the epidermis. The overall pattern of starch

content was similar to that of lipids in that the epidermis consistently had lower starch levels than the other two areas of the tepal.

Among fragrant genotypes, the amount of lipid in the sub-epidermal and central areas of the tepal was greatest in hybrid A491 (Appendix 4). Among non-fragrant genotypes, the amount of lipid in the tepal was greatest in hybrid A494 (Appendix 4).

Figure 5.6 Gas chromatograms of solvent extracts from spadices of (A) fragrant *Anthurium* hybrid, UH1299 and (B) non-fragrant hybrid, UH1269

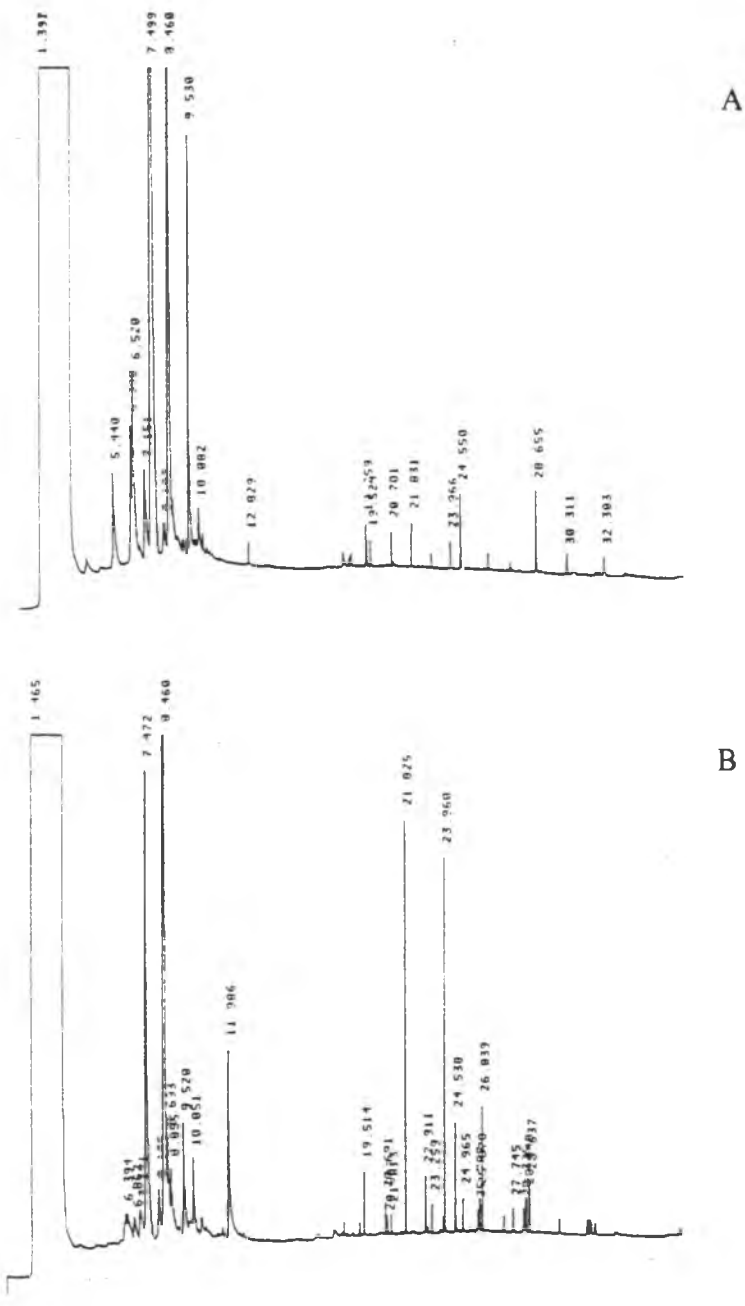

Figure 5.7 Gas chromatograms of solvent extracts of (A) tepals and (B) pistils of fragrant *Anthurium* hybrid, UH1299.

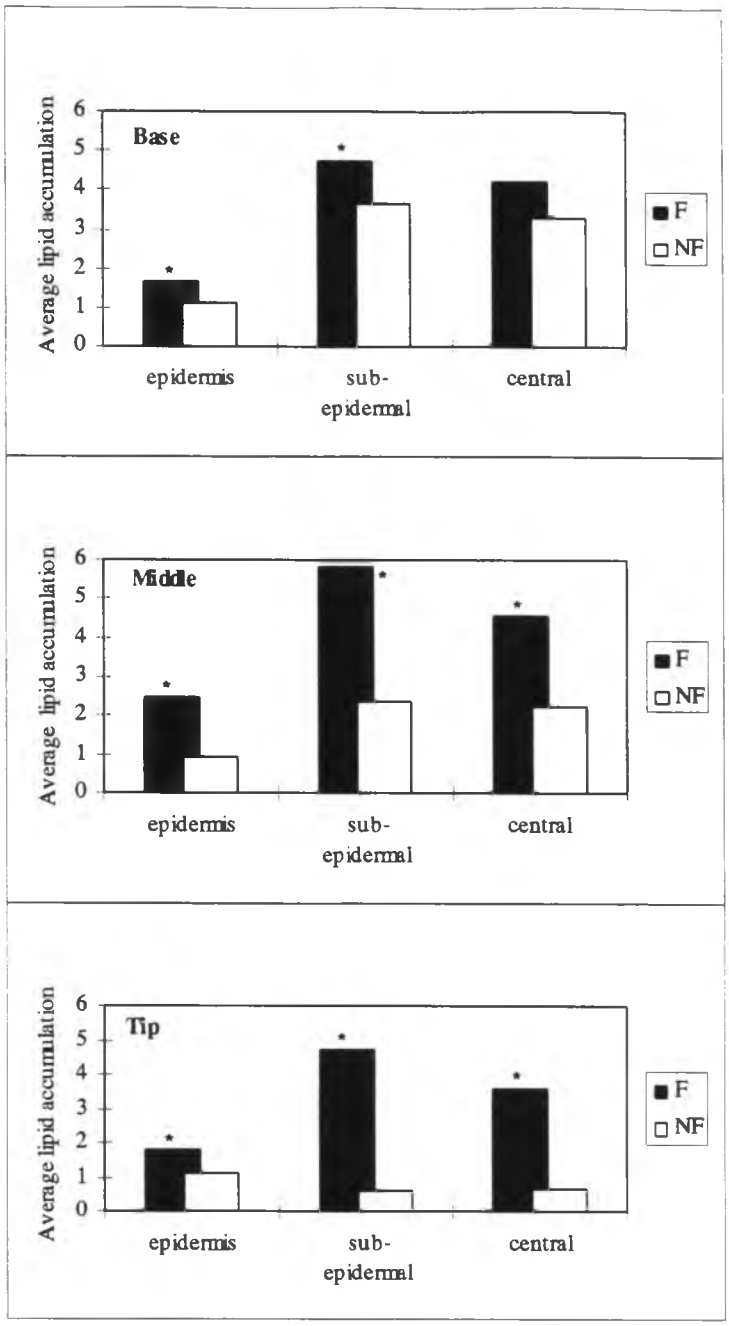

Figure 5.8 Average lipid accumulation in the epidermis, sub-epidermal and central region of tepals from three zones of three fragrant and three non-fragrant *Anthurium* spadices. * Mann-Whitney test was significant at 0.05, F = fragrant spadices, NF = non-fragrant spadices.

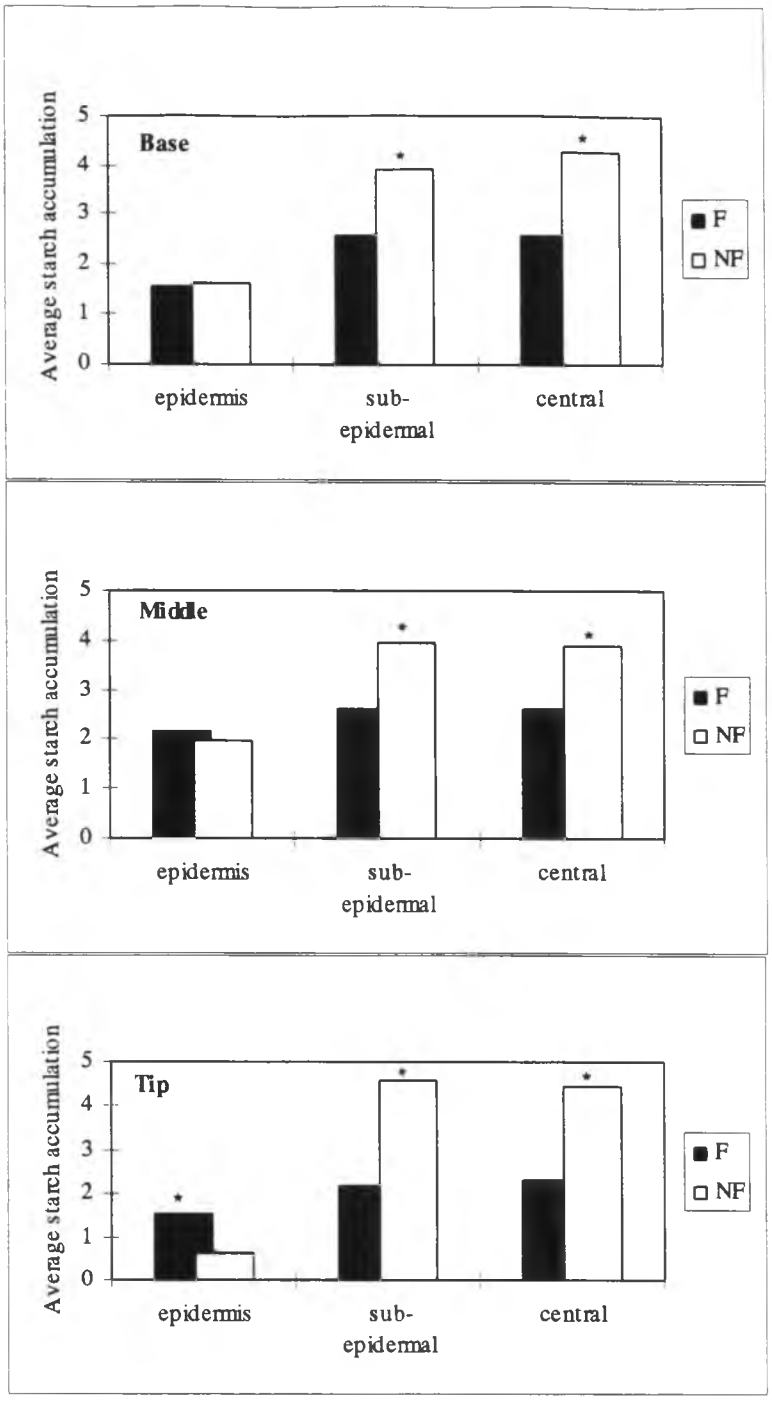

Figure 5.9 Average starch accumulation in the epidermis, sub-epidermal and central region of tepals from three zones of three fragrant and three non-fragrant *Anthurium* spadices. * Mann-Whitney test was significant at 0.05, F = fragrant spadices, NF = non-fragrant spadices.

5.5 Discussion

Chemical analysis of tepals, pistils and stamens obtained from a fragrant spadix showed that only tepals and pistils contained volatile monoterpenes, the non-fragrant spadix did not produce fragrance volatiles. They indicated that source of scent production should have come from these two structures. Histological comparison of fragrant and non-fragrant tepals and pistils showed that storage compounds in tepals differed. Fragrant tepals accumulated greater amount of lipid and lesser amount of starch than non-fragrant ones. The presence of lipids might be related to volatile compounds in the fragrant spadix. In *Sauromatum guttatum*, osmophilic materials were identified as sesquiterpenes (Skubatz *et al.*, 1995). These compounds were found in the cells before scent emission. Soon after scent was released, these compounds disappeared.

Unlike other aroids in which heat and scent production occur simultaneously (Kite, 1995, Knutson, 1974, Skubatz and Meeuse, 1993), no heat production was detected during the time of scent emission. In *Anthurium*, volatile compounds might diffuse through epidermal cell as thought to be that case in rose (Morris, 1984). Lipid and starch levels were lowest in the epidermis of all spadix zones. One would not expect storage reserve in the epidermis if it was the secretory tissue in scent production. Starch was detected in lower amounts in the epidermis than in adjacent cells of the *Cryptocoryne ciliata* spathe (Vogel, 1990) and in the fragrant *Restrepia* (Orchidaceae) petal (Pridgeon and Stern, 1983).

In regard to flower development, the amount of lipid and starch was slightly greater in the middle receptive zone tepals of the spadix compared to the basal and tip zones. The cells at the base might have depleted their storage products due to their advanced state of development, while those at the tip might be immature, with storage products at sub-maximal levels. A similar phenomenon was found in *Restrepia*, where young unopened petals had less starch than mature petals during anthesis (Pridgeon and Stern, 1983).

Quantitative gene action might be involved in fragrance inheritance. The amount of lipid in the tepal of the hybrid, A491 derived from two fragrant parents, *A. antioquiense* × *A. amnicola* was greater than that of *A. antioquiense*. If there were additive gene action, the amount of lipid present in the hybrid would be expected to be greater than either of the parents. All the genotypes in this study except for UH1750 had *A. antioquiense* in their background. The differences in the amounts of lipid in the specimens might be genetic because fragrance was found to be a transmissable trait (Chapter 3).

However, it is possible that other factors are also involved in determining scent production. For example, a critical enzyme involved in scent production may be absent in non-fragrant genotype. Even though a greater amount of lipid was found in the tepals of hybrid A494, derived from *A. andraeanum* × *A. antioquiense*, no scent was detected.

Chemical analysis revealed that several monoterpenes were major components of scented *Anthurium*. Monoterpenes found in fragrant *Anthurium* are derived from the mevalonic acid pathway. This pathway initiates from acetyl CoA, which is a product of fatty acid or starch breakdown (Taiz and Zeiger, 1991). Thus, lipids found in tepals might

be a direct source for monoterpene synthesis. However, acetyl CoA could be derived from starch breakdown for use in the Krebs cycle. Further examination of the literature might shed light in which is the preferred energy efficient pathway for monoterpene synthesis.

In summary, this was the first study in which chemical and histological comparisons were done between fragrant and non-fragrant aroid spadices. No heat production was detected at the time of scent emission. The tepal appears to be the floral structure associated with scent production in *Anthurium*. Although both lipids and starch were present in fragrant and non-fragrant spadices, the amount of lipids was significantly greater in fragrant spadices. Lipids were probably used preferentially for scent production while starch was not. These results support the postulate that high levels of lipids are associated with scent production. Further studies need to be done in order to understand fragrance biosynthesis in tepals of *Anthurium*.

References

Baker, J. R. 1958. *Principles of biological microtechnique*. John Wiley & Sons Inc., New York. 357 pp.

Bermadinger-Stabentheiner, E. and A. Stabentheiner. 1995. Dynamics of thermogenesis and structure of epidermal tissues in inflorescences of *Arum maculatum*. *New Phytol.* 131: 41-50.

Bown, D. 1988. *Aroids*. Timber Press, Portland, Oregon. 256 pp.

Bronner, R. 1975. Simultaneous demonstration of lipids and starch in plant tissue. *Stain Tech.* 50:1-4

Croat, T. B. 1980. Flowering behavior of the neotropical genus *Anthurium* (Araceae). *Amer. J. Bot.* 67: 888-904.

Croat, T. B. 1992. Species diversity of Araceae in Colombia: A preliminary survey. *Ann. Missouri Bot. Gard.* 79: 17-28.

Feder, N. and T. P. O'Brien. 1968. Plant microtechnique: some principles and new methods. *Amer. J. Bot.* 55:123-142.

Higaki, T., H. P. Rasmussen, and W. J. Carpenter. 1984. A study of some morphological and anatomical aspects of *Anthurium andraeanum* Lind. College of Tropical Agriculture and Human Resources, University of Hawaii. Research Series 030.

Kite, G. C. 1995. The floral odour of *Arum maculatum*. *Biochem. Sys. Ecol.* 32: 343-354.

Knutson, R. M. 1974. Heat production and temperature regulation in eastern skunk cabbage. *Science* 186:746-747.

Matsumoto, T. K., A. R. Kuehnle, and D. T. Webb. 1995. Improvements for observing *Anthurium* (Araceae) floral structures by light microscopy. *Biotech. & Histochem.* 70: 205-210.

Morris, E. T. 1984. *Fragrance*. Charles Scribner's Sons, New York. 304 pp.

Pridgeon, A. M. and W. L. Stern. 1983. Ultrastructure of osmophores in *Restrepia* (Orchidaceae). *Amer. J. Bot.* 70:1233-1243.

Skubatz, H., D. D. Kunkel, and B. J. D. Meeuse. 1993. Ultrastructural changes in the appendix of the *Sauromatum guttatum* inflorescence during anthesis. *Sex. Plant Repro.* 6: 153-170.

Skubatz, H. and B. J. D. Meeuse. 1993. Energy loss in tissue slices of the inflorescence of *Sauromatum guttatum* (Schott) analysed by microcalorimetry. *J. Exp. Bot.* 44:493-499.

Skubatz, H. D. D. Kunkel, J. M. Patt, W. N. Howald, T. G. Hartman and B. D. Meeuse. 1995. Oleic acid and its positional isomer, cis-vaccine acid, in the appendix of *Sauromatum guttatum* during anthesis. *Proc. Nat. Acad. Sci.* 92:10084-10088.

Sprent, P. 1993. *Applied nonparametric statistical methods*. Chapman & Hall, Suffolk. 342 pp.

Taiz, L. and E. Zeiger. 1991. *Plant physiology*. The Benjamin/Cummings Publishing Co., Inc., Redwood City, California 559 pp.

Vogel, S. 1990. *The role of scent glands in pollination*. A. A. Balkema, Rotterdam. 202 pp.

CHAPTER 6

ANTHURIUM FRAGRANCE BREEDING

6.1 Abstract

Approaches to obtain useful information in fragrant *Anthurium* breeding were described. Several species and hybrids have good potential to be used as parental plants. It was found that genetic control of the fragrance trait was not by a single gene. Results from chemical and histological studies supported involvement of more than one gene in fragrance production in *Anthurium.*

6.2 Introduction

Little is known about breeding for flower fragrance. Despite a wealth of anecdotal evidence, genetics of scent have been analyzed for only rice and gladiolus flowers (Dhulappanavar 1976; McLean 1933; McLean 1938; Nagaraju *et al.*, 1975). In rice, a single recessive gene was responsible for fragrance. In gladiolus, results were inconclusive as the trait appeared to be quantitatively inherited.

Why so little is reported for ornamentals might be due to proprietary concerns of private breeding companies or to the complexity of the flower fragrance itself. Several inquiry letters were sent out to various breeders, but only two replied. The rose breeder in

France's Meilland International could not divulge information of the company (S. Gudin, Domaine de Saint Andre', 1996, personal communication). Another rose breeder in the United States' Jackson & Perkin Company stated that the heredity of fragrance in rose is complex. Progenies from crosses between fragrant roses sometime yielded non-fragrant roses. On the other hand, crosses between non-fragrant roses could yield fragrant progenies. He also suggested that the fragrance trait is derived from several genes probably located on several chromosomes (Zary Keith, Jackson & Perkin Company, 1996, personal communication).

An approach to studying the heredity of flower fragrance is proposed in this chapter by using fragrant *Anthurium* as an example.

6.3 Approach

In order to conduct a breeding program for fragrant *Anthurium*, some basic knowledge is needed. One needs to:

1) Survey the availability of fragrant species or hybrids with the potential for use as parental plants.

2) Determine the genetic control of the fragrance character in species and hybrids.

3) Analyze chemical components of fragrance to determine the types of compounds involved.

4) Identify structures associated with scent production.

Details of each approach are described below.

6.3.1 Survey

The floral scent of germplasm should be assessed from several perspectives in order to set objectives and experimental design of the breeding program.

1) What is the scent quality?

2) Does the plant emit scent at different times of day?

3) Is there a developmental stage for scent production, i.e. pistillate or staminate?

4) What is the fragrance life of scent on unharvested or harvested flowers?

5) How is scent production affected by environment?

6) How do environmental conditions affect detection of scent by the human nose?

Words used in describing flower scent are very important for communication and marketing. Scent description should represent the scent that the plant produces. However, if no standard is used, the description may vary according to experience and knowledge of the person who evaluates scent. Description based on standard compounds aids the breeder and others to communicate with a meaningful and consistent vocabulary.

Training for lexicon development can be done. In addition to having well trained panelists and a qualified panel leader, availability of flowers for each cultivar may be a limiting factor for lexicon development. At least 10 panelists are needed and individual panelists should be accessible and evaluate flowers as needed. Test flowers must be abundant and samples must have the same stage of development for consistency. Chemical analysis might alleviate some ambiguities in describing flower scent. Recently, an electronic nose was invented, however, the technology is quite limited (Breer, 1997).

In a survey of *Anthurium* plants, it was found that a majority (77%) of scented plants produced scent at the pistillate stage and about 45% produced scent in the morning. This character of female flowers emitting scent in the morning time, seems to be heritable. The species *A. amnicola*, *A. antioquiense* and *A. armeniense*, employed as parental plants in breeding program, produce scent only in the morning and at the pistillate stage. A majority of progenies derived from these species also produce scent in the morning and at the pistillate stage (Chapter 2).

Scent emission occurs on both unharvested and harvested flowers. In major cut flowers such as rose, carnation and rose, scent lasts for the life of flowers. In *Anthurium*, while fragrance life of unharvested inflorescences lasted from 3-4 days up to 2-3 weeks, scent on harvested inflorescences lasted only 1-2 days.

Temperature and humidity play important role in scent production and detection (Allen, 1980; Bouquet, 1968; Burbott and Loomis, 1967; Carruth, 1992; Harkness, 1992; Tingey *et al.*, 1979). In *Anthurium*, strong scent was detected at temperature of 25 C and relative humidity of a range 85-90%.

Observing scent emission behavior of both parents and their progenies will be very useful for a breeding program. Certain emission times might be linked with certain types of scent. For example, trends in our data suggest that sweet or minty scent might be produced only in the morning whereas pine is produced all day.

However, variation occurred among accessions of the same species. In the case of *A. formosum*, one accession (A507) emitted scent in the morning whereas the other accession (A291) produced scent all day. In *A. sanctifidense*, accession no. 503 produced

minty scent whereas in accession no. 592 produced no scent. Due to variation within the same species, only the plant with a desirable characteristics should be introduced into a breeding program.

6.3.2 Conventional Breeding

Cross-pollination with selected parents is the primary method to test whether the trait of interest is qualitative or quantitative. Most of the qualitative traits such as flower color, skin type and plant height are controlled by one or two genes, whereas quantitative traits such as yield and size, are governed by polygenes (Simmond, 1987). In terms of fragrance, if this trait is inherited as a single gene, a cross between fragrant and non-fragrant parents should yield either fragrant or non-fragrant progenies in the F_1 generation. However, if the fragrant parent is in a heterozygous condition for the gene, the progenies in the F_1 generation will segregate into 1 : 1, fragrant : non-fragrant. However, if segregation does not occur as a 1 : 1 ratio, in the F_1 generation, the fragrance trait might be governed by more than one gene and/or be inherited quantitatively.

In this first study on fragrance genetic inheritance, results showed that the fragrant trait most likely is quantitative, not qualitative. Even though fragrance is heritable in *Anthurium*, not all-scented species of *Anthurium* can be used in hybridization program due to incompatibilities with breeding lines. Only the compatible species for example, those belonging to sections Calomystrium and Porphyrochitonium are crossable (Kamemoto and Kuehnle, 1996). The section Calomystrium includes the fragrant species *A. lindenianum*

and *A. nymphaefolium*. The section Porphyrochitonium includes the fragrant *A. amnicola* and *A. antioquiense*.

6.3.3 Chemical Analysis

In *Anthurium*, chemical profiles of seven species were reported (Chapter 4). Each species showed a specific pattern. The common compound found in the species was limonene. This compound was found in species that produced floral and minty scent. Comparison of chemical profiles between parental plants and their progenies showed similarities among presence and relative proportions of monoterpenes. In addition, when chemical profiles of unknown hybrids were compared with potential parents, it appears that chemical profiles can be used along with morphology to trace and determine relatedness for an *Anthurium* species and/or its hybrid. In addition to possible correlation with emission time, type of compound might be related to type of pollinator. Currently, the relationship between scent emission and pollinators in *Anthurium* is not well understood (Croat, 1980).

Classifications of Anthurium, particularly species belong to sections Belolonchium, Pachyneurium and Xialophyllium, is still unsettled (T. Croat, 1997, personal communication). Chemical analysis might be a useful tool in sectional classification. Relationships between chemical constituents and classification of plants have been studied (Sivarajan, 1991). Different ranks of taxa can be addressed with chemical profiles. Species of certain genera such as *Vetiveria* and *Cymbopogon* were characterized according to the presence of terpenes (Sivarajan, 1991). In the orchid genus *Coryanthus*, chemical profiles

were used to aid species classification (Gerlach and Schill, 1989). Similar chemical profiles were found in the closest related species whereas different chemical profiles were seen among non-related species. In *Ocimum basilicum* (Grayer *et al.*, 1996), infraspecific classification could be done using chemical profiles.

Chemical analysis also aids understanding of the number of genes that control biosynthesis of certain compounds, especially compounds that do not share any common pathway. This situation might parallel that of flower color. Kamemoto *et al.* (1988) found that there are two major genes that control color in anthurium flowers. The combination of these two genes regulates the production of two major compounds, cyanidin 3-rutinoside and pelargonidin 3-rutinoside. In floral fragrance, the greater the number of chemical compounds found, the greater the number of genes that might regulate fragrance production.

6.3.4 Histological Study

Scent production can occur in different parts of plants. In *Anthurium*, it was found that the tepal was one site associated with scent production (Chapter 5). Comparison between amounts of lipids and starch present in fragrant and non-fragrant lines showed that tepals of fragrant samples contained greater amount of lipids. The involvement of lipids in scent production in *Anthurium* is not yet known. Further study on enzymes involved in fragrance production might indicate the subcellular location of fragrance synthesis as was done in the orchid *Stanhopea anfracta* (Curry, 1987). Enzymes involved in the mevalonic pathway in this orchid were found in the smooth endoplasmic reticulum,

and in between inner and outer mitochrondrial membranes. One possible explanation for why scent was not detected from non-fragrant spadices might be due to a lack of one or more enzymes. These enzymes might be essential in converting lipid or starch to volatile compounds (Appendix 6).

6.4 Application to *Anthurium* Fragrance Breeding

Recently, several cultivars of anthuriums were identified as scented despite no mention of this attribute in the market (Chapter 2). The species originally introduced for cultivation, *A. andraeanum*, had a spathe color of red-orange (Kamemoto and Kuehnle, 1996). It was suggested that other cultivated anthuriums with pink or white spathes resulted from intercrossing of *A. andraeanum* with *A. lindenianum* and *A. nymphaefolium*. The latter two species had white spathes and were fragrant as reported in Chapter 2 of this dissertation. Progenies derived from crosses between *A. andraeanum* and *A. lindenianum* or *A. nymphaefolium* segregated with various colors (Kamemoto *et al.*, 1988; Chapter 3). It is highly likely that scent was also introduced into hybrids in this fashion.

The scent character has been generally neglected in *Anthurium* breeding. In the past few decades, breeding has focussed on novel color of spathe and spadix, shape, disease resistance, high yield and good keeping quality. However, with the recent popularity of fragrant plants in the market (Barletta, 1995; DLO, 1997), fragrance in *Anthurium* is expected to enhance its marketability (Halloran and Kuehnle, in preparation).

Kamemoto and Kuehnle (1996) mentioned that scent was heritable in *Anthurium* but inheritance of this character was never studied.

A. antioquiense was used extensively in the University of Hawaii breeding program because of its promising characteristics and tolerance to bacterial blight (Kamemoto and Kuehnle, 1996). From the survey results (Chapter 2), no scent was detected from this species while in the greenhouse environment. However, a strong scent was produced from several progenies derived from *A. antioquiense* such as UH1299 and cross 768 (Chapter 2). Moreover, when several flowers of *A. antioquiense* were cut and brought to the laboratory in the morning for chemical analysis, a very faint minty scent was detected by eight people, but not by the project leader until much later in the day. Results from chemical analysis of solvent extracts of spadices of *A. antioquiense* revealed several volatile compounds including 1,8-cineole, benzyl alcohol, benzyl acetate and α-terpineol. In histochemical study, lipids and starches were found in the tepal of *A. antioquiense*. Both compounds were found to be primary sources for scent production in several aroid species (Skubatz *et al.*, 1993; Vogel, 1990). Results from chemical analysis and histochemical study indicated that *A. antioquiense* is a fragrant species, even though the amount of scent produced might be so little as to be undetectable in the greenhouse environment.

Results from histological study showed that lipid were a major compound stored in fragrant *Anthurium* (Chapter 5). Monoterpenes comprised the bulk of volatile compounds identified in fragrant *Anthurium* species (Chapter 4). Acetyl CoA, which can be derived from either fatty acid breakdown or glycolysis, is the key precursor in monoterpene

biosynthesis (Taiz and Zeiger, 1991). The main source for monoterpene biosynthesis in *Anthurium* is not known.

A seven-member expert panel was set up in 1994 to develop a lexicon to evaluate scented *Anthurium* inflorescences. Harvested inflorescences of *A. armeniense*, 'Lady Beth', A624, A626, A628, RS1361-1, 649-7, 1061-1, 1061-11 and 1061-15 were brought into the conference room of the Department of Horticulture. Panelists sniffed and evaluated the scent of individual inflorescences. Terms and definitions of fragrance were provided (Appendix 7). Individual panelists judged and wrote down the appropriate type of fragrance that represented the inflorescence scent. After all the inflorescences were evaluated, terms used by individual panelists were discussed and consensus was reached (Table 6.1).

Due to insufficient numbers of flowers and timing of flowering and evaluation by the full panel could not be done. Evaluation of the experimental inflorescenses was conducted by the author using the lexicon developed from the panel.

However, a preliminary study to confirm the lexicon was done. A total of 127 persons, 68 males and 59 females, participated in this study. The majority of participants were aged below 20. An evaluation form was given to each person (Appendix 5) who then sniffed flowers and checked category boxes based on his/her perception. The test was conducted from 9:00 am - 2:00 pm on cut flowers in vases. Results are presented in Table 6.2.

Table 6.1 Terms used by an expert panel in describing scent of harvested *Anthurium* inflorescences.

Harvested *Anthurium* inflorescences	Scent description
A. armeniense	sweet
'Lady Beth'	sweet
A624	sweet, floral
A626	floral
A628	floral
RS1361-1	minty
649-7	sweet, floral
1061-1	fruity
1061-11	fruity
1061-15	fruity

Table 6.2 Evaluation of five scented anthurium hybrids, *A.* 'ARCS', *A.* 'Lady Beth', UH1299, 633-41 and 649-7, compared with the descriptors used in Chapter 2.

Flower	Descriptor used	Frequency of category selection*				
		None	Minty	Floral	Sweet	Spicy
A. 'ARCS'	minty	36	45	7	15	38
A. 'Lady Beth'	sweet, floral	2	9	68	57	6
UH1299	sweet, floral	29	18	36	39	22
633-41	minty	8	55	7	10	52
649-7	sweet, floral	8	11	70	43	8

* Some flowers were included in more than one category. Scoring was done by 127 people during a College of Tropical Agriculture and Human Resources Educational Fair Day on April 18, 1997. More than one descriptor was assigned a flower by some evaluators.

Results showed that the majority of people could distinguish two general categories, namely minty - spicy and floral - sweet (Table 6.2). In *A.* 'ARCS' and 633-41, minty and spicy were chosen to describe flower fragrance. In *A.* 'Lady Beth' and 649-7, a majority chose floral and sweet to represent the scent of these flowers. In the case of UH1299, it appeared that the scent of this flower was complex as people could not readily distinguish its scent. However, evaluation done by participants at CTAHR Educational Fair Day showed that the description of anthurium scent used in Chapter 2 was acceptable.

6.5 Concluding Remarks

Varieties of fragrance were found in *Anthurium*. Some of fragrant species may possibly be used in a breeding program. However, compatibility needs to be taken into account. Fragrance life on the unharvested flower suggested that breeding fragrance anthurium should move towards potted plants. While the fragrance trait is heritable, genetic control of this trait is not well understood. This trait is not linked with white color. Chemical analysis revealed that compounds found in scented *Anthurium* are very common in floral scent in general. Lipid and starch were found in both fragrant and non-fragrant spadices. At least one structure, the tepal, was a site of scent production in *Anthurium*.

Even though a major first step towards understanding fragrance inheritance in *Anthurium* was taken in this study, several questions need to be answered in the future. Combining genetic study with more extensive chemical analysis of parental species and hybrids will provide more complete information on which biosynthetic or regulatory genes are passed on from parental plants to progenies. Histological work at the intracellular level will be very helpful for understanding the process of scent production in *Anthurium* and may identify early limiting steps. The final challenge that remains is the combination of scent with other desirable characters, such as attractive flower colors and shapes and adequate flower yield, needed in successful varietal development.

References

Allen, E. F. 1980. Rose scent - A quantitative study. *The Rose Ann.* pp. 58-61

Barletta, A. 1995. Scent makes a come back. *FloraCul. Inter.* pp. 23-25.

Bouquet, A. 1968. Perfumes of the rose. *Amer. Rose. Ann.* 53:107-111.

Breer, H. 1997. Sense of smell: signal recognition and transduction in olfactory receptor neurons. *(In)* E. Kress-Rogers, ed. *Handbook of biosensors and electronic noses: medicine, food and the environment.* CRC Press, Inc., New York. pp. 521-532.

Burbott, A. J. and W. D. Loomis. 1967. Effects of light and temperature on the monoterpenes of peppermint. *Plant Physiol.* 42:20-28.

Carruth, T. 1992. The fickle finger of fragrance. *Amer. Rose Ann.* 77:24-25.

Croat, T. B. 1980. Flowering behavior of the neotropical genus *Anthurium* (Araceae). *Amer. J. Bot.* 67: 888-904.

Curry, K. J. 1987. Initiation of terpenoid synthesis in osmophores of *Stanhopea anfracta* (Orchidaceae): a cytochemical study. *Amer. J. Bot.* 74: 1332-1338.

Dhulappanavar, C. V. 1976. Inheritance of scent in rice. *Euphy.* 25: 659-662.

DLO. 1997. Fragrance research. *FloraCul. Inter.* p. 32.

Gerlach, G. and R. Schill. 1989. Fragrance analyses, an aid to taxonomic relationships of the genus *Coryanthes* (Orchidaceae). *Plant Sys. Evol.* 168: 159-165.

Grayer, R. J., G. C. Kite, F. J. Goldstone, S. E. Bryan, A. Paton and E. Putievsky. 1996. Infraspecific taxonomy and essential oil chemotypes in sweet basil, *Ocimum basilicum*. *Phytochem.* 43: 1033-1039.

Harkness, P. 1992. Roses for noses. *Amer. Rose* pp. 15-17.

Kamemoto, H., R. Y. Iwata and M. Muratani. 1988. Genetics of the major spathe colors in anthuriums. Hawaii Int. Trop. Agr. and Human Res. Ser. 56.

Kamemoto, H. and A. R. Kuehnle. 1996. *Breeding anthuriums in Hawaii*. University of Hawaii Press, Honolulu, Hawaii. 132 pp.

McLean, F. T. 1933. The inheritance of fragrance in gladiolus species crosses. *The New York Bot. Gard.* 34: 73-80.

McLean, F. T. 1938. A genetic analysis of the inheritance of fragrance in gladiolus. *Bul. Torr. Bot. Club* 65: 181-197.

Nagaraju, M., D. Chaudhary and M. J. B. Rao. 1975. A simple technique to identify scent in rice and inheritance pattern of scent. *Cur. Sci.* 44: 599.

Simmond, N. W. 1987. *Principles of crop improvement*. 4th ed. Longman Scientific & Technical, London. 408 pp.

Sivarajan, V. V. 1991. Sources of taxonomic characters. (*In*) N. K. P. Robson, ed. *Introduction to the principles of plant taxonomy*. Cambridge University Press, Melborne, Australia pp.170-197.

Skubatz, H., D. D. Kunkel and B. J. D. Meeuse. 1993. Ultrastructure changes in the appendix of the *Sauromatum guttatum* inflorescence during anthesis. *Sex Plant Reprod.* 6:153-170.

Taiz, L. and E. Zeiger. 1991. *Plant physiology*. The Benjamin/Cummings Publishing Company, Inc., Redwood City, California. 559 pp.

Tingey, D. T., M. Manning, L. C. Grothaus and W. F. Burns. 1979. The influence of light and temperature on isoprene emission rates from live oak. *Physiol. Plant* 47:112-118.

Vogel, S. 1990. *The role of scent glands in pollination*. A. A. Balkema, Rotterdam. 202 pp.

APPENDIX I

Table 1 *Anthurium* crosses made during 1992-3 for fragrance genetic study.

Cross no.	Female parent	F/NF	Male parent	F/NF	remarks
1172	UH585	NF	*A. lindenianum* (A170)	F	
1180	'Trinidad'	F	*A. lindenianum* (A220-2)	F	
1181	'Trinidad'	F	*A nymphaeifolium* (A213-2)	F	
1182	UH566	NF	*A. lindenianum* (A220-2)	F	
1183	A38	NF	*A. lindenianum* (A220-2)	F	
1184	692-48	F	*A. lindenianum* (A220-2)	F	
1185	UH818	NF	*A. lindenianum* (A220-2)	F	
1186	UH585	NF	*A lindenianum* (A220-2)	F	
1188	A558	NF	*A. lindenianum* (A220-2)	F	
1195	A494	NF	RS1361-1	F	
1196	UH585	NF	*A lindenianum* (A220-2)	F	
1197	UH186	NF	*A. lindenianum* (A220-2)	F	
1198	UH185	NF	*A. lindenianum* (A220-2)	F	
1199	'Calypso'	NF	*A. lindenianum* (A170)	F	
1200	383	NF	*A. lindenianum* (A220-2)	F	
1201	A99	NF	*A. concinnatum* (A212)	F	
1202	'Kozohara'	NF	*A. lindenianum* (A220-2)	F	
1203	'Paradise Pink'	NF	*A. lindenianum* (A220-2)	F	
1204	'Fujii Light Pink'	NF	RS1361-1	F	
1205	A38	NF	*A. lindenianum* (A220-2)	F	
1206	A167	NF	*A. concinnatum* (A212)	F	
1207	A67	NF	*A. lindenianum* (A220-2)	F	
1208	A99	NF	*A. concinnatum* (A220-2)	F	
1209	UH585	NF	*A. lindenianum* (A170)	F	
1210	'Marian Seefurth'	NF	*A. lindenianum* (A220-2)	F	
1211	UH712	NF	*A. lindenianum* (A220-2)	F	
1212	A167-2	NF	*A. lindenianum* (A220-2)	F	
1213	'Ellison Onizuka'	NF	*A. armeniense* (A382)	F	
1214	*A. lindenianum* (A220-2)	F	1047-227	NF	
1216	649-7	F	UH1311	NF	
	UH711	NF	RS1361-1	F	dead
	633-41	F	A151-2	NF	dead
	'ARCS'	F	UH566	NF	dead
	'Marian Seefurth'	NF	*A. lindenianum* (A220-2)	F	dead
	649-7	F	Murayama Red (A159)	NF	dead
	649-7	F	Princess Lily	NF	dead
	A99	NF	*A. lindenianum* (A170)	F	dead
	A. lindenianum (A220-2)	F	*A. lindenianum* (A220-2)	F	dead
	UH567	NF	*A. armeniense* (A382)	F	dead
	UH948	NF	*A. lindenianum* (A220-2)	F	dead
	Obake	NF	*A. lindenianum* (A170)	F	BB
	Obake	NF	*A. lindenianum* (A185)	F	BB
	A103	NF	*A. lindenianum* (A220-2)	F	BB
	649-2	F	A146	NF	BB

F = Fragrant *Anthurium*, NF = non-fragrant *Anthurium*, BB = died due to bacterial blight.

APPENDIX 2

Table 1 Common compounds retained longer than 50 min, in GC-MS results of solvent extracts of *Anthurium* spadices.

GC retention time (min)	Compounds
57.66	benzyl benzoate
61.24	heneicosane*
62.75	hexadecane*
63.31	decane*
64.44	nonadecane*
66.68	pentacosane*
70.03	eicosane*

* wax

APPENDIX 3

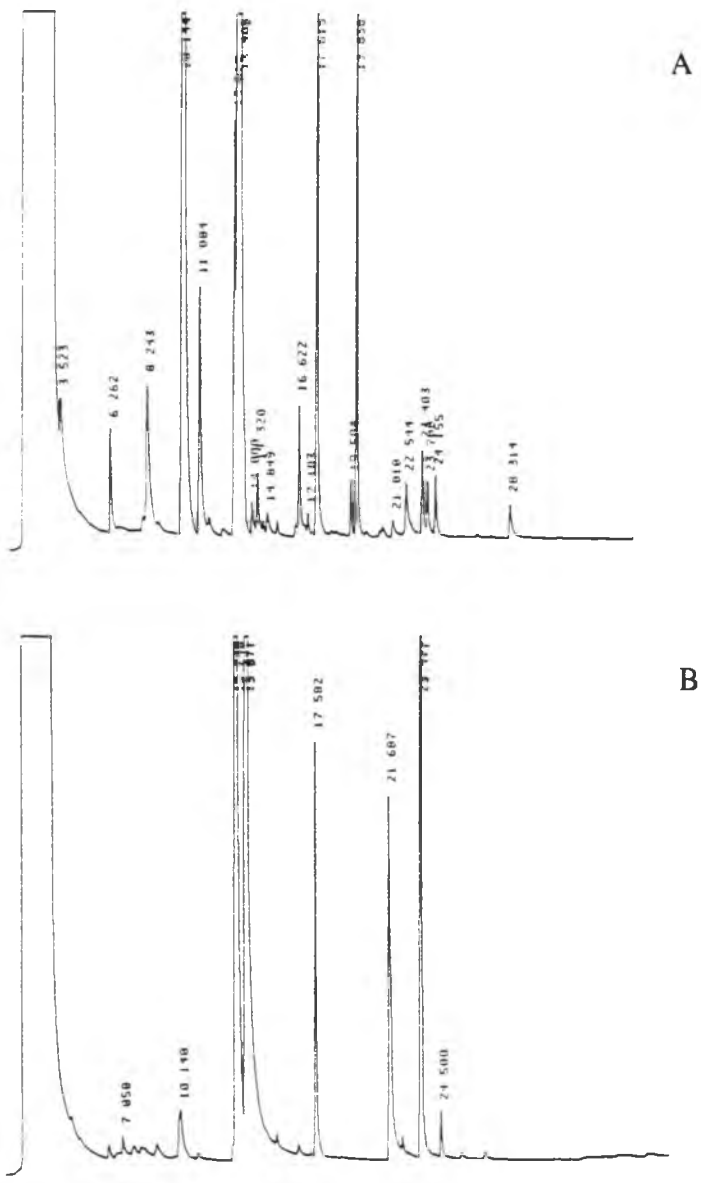

Figure 1 Chromatograms of *A.* 'Leilani' samples obtained via headspace (A) and solvent extraction (B).

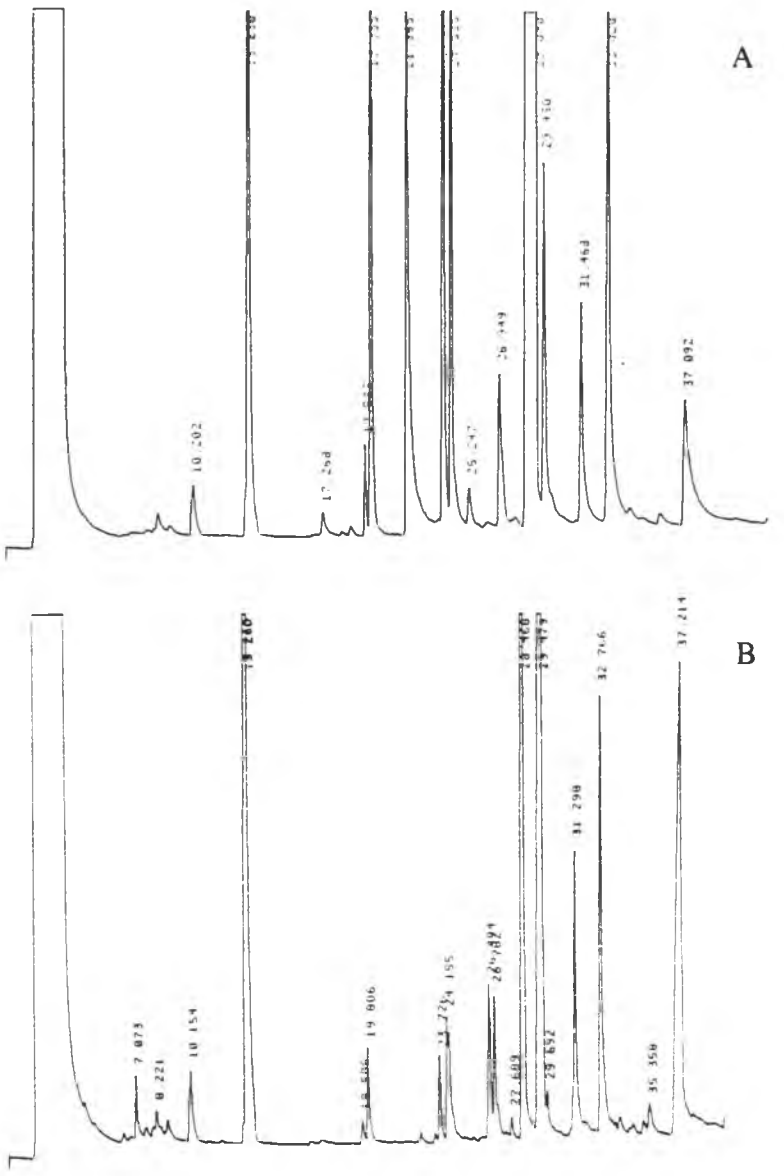

Figure 2 Chromatograms of RS1316-1 (A) and 633-41 (B) samples obtained from solvent extraction.

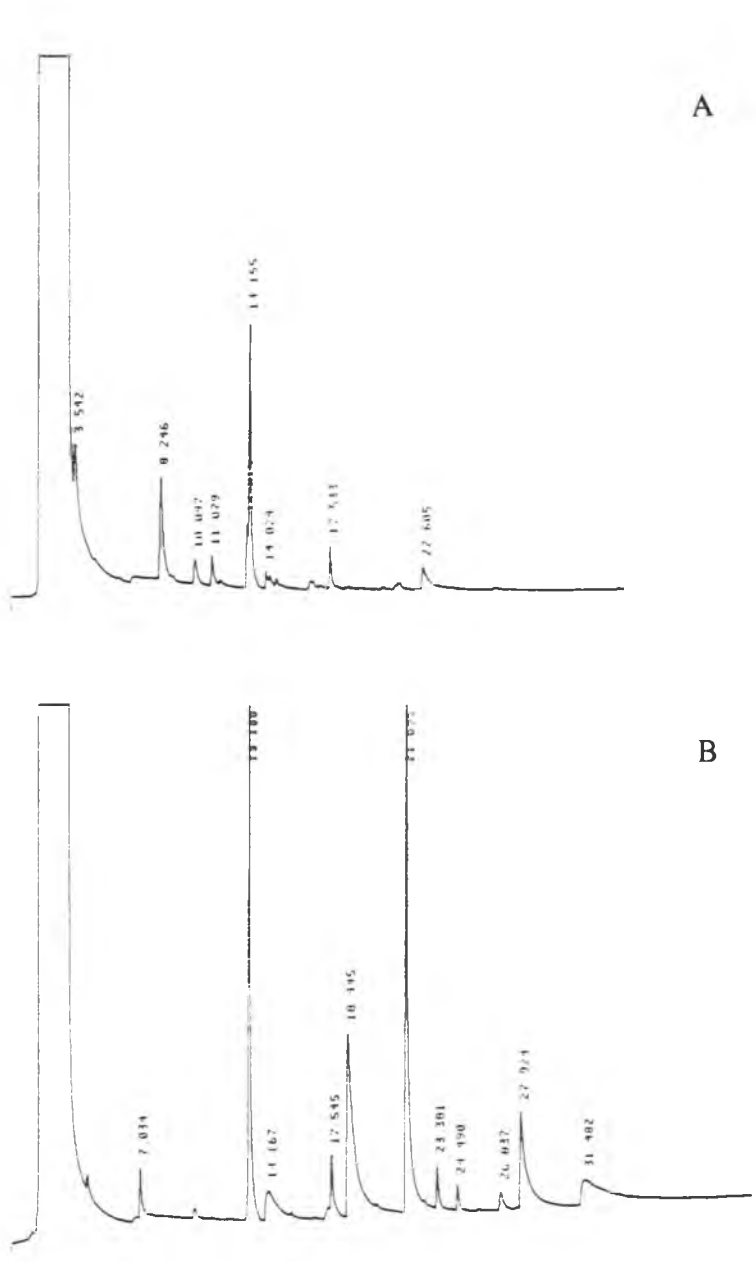

Figure 3 Chromatograms of A626 (A) and 1159-2 (B) samples obtained via headspace.

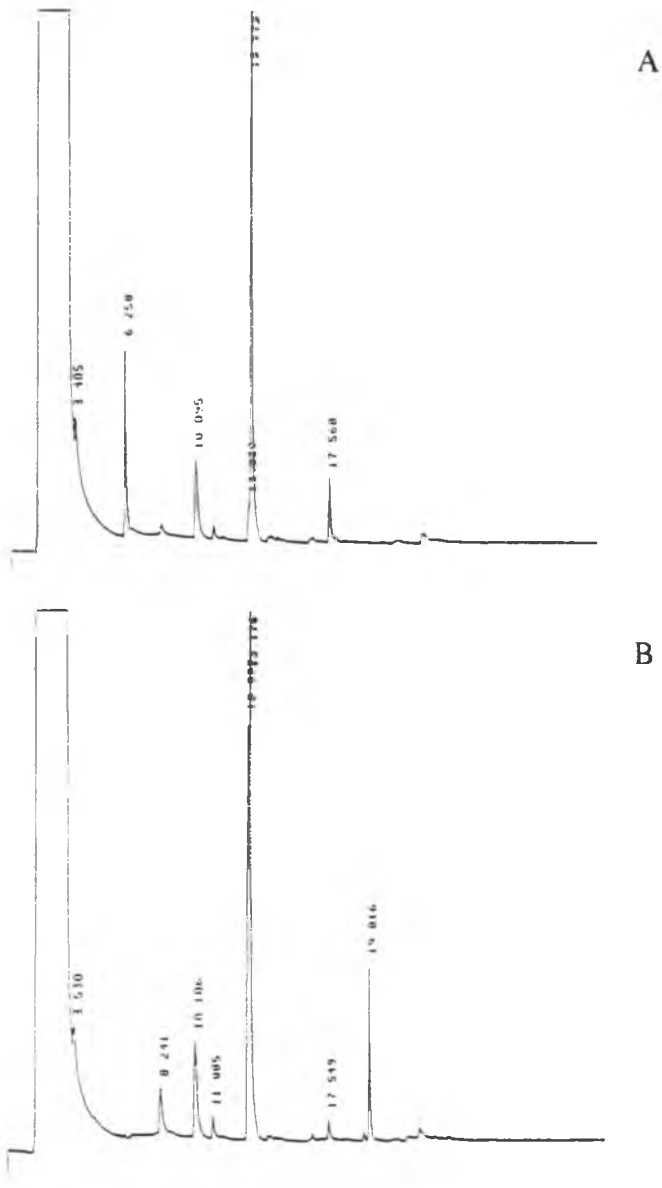

Figure 4 Chromatograms of 649-2 (A) and 649-7 (B) samples obtained via headspace.

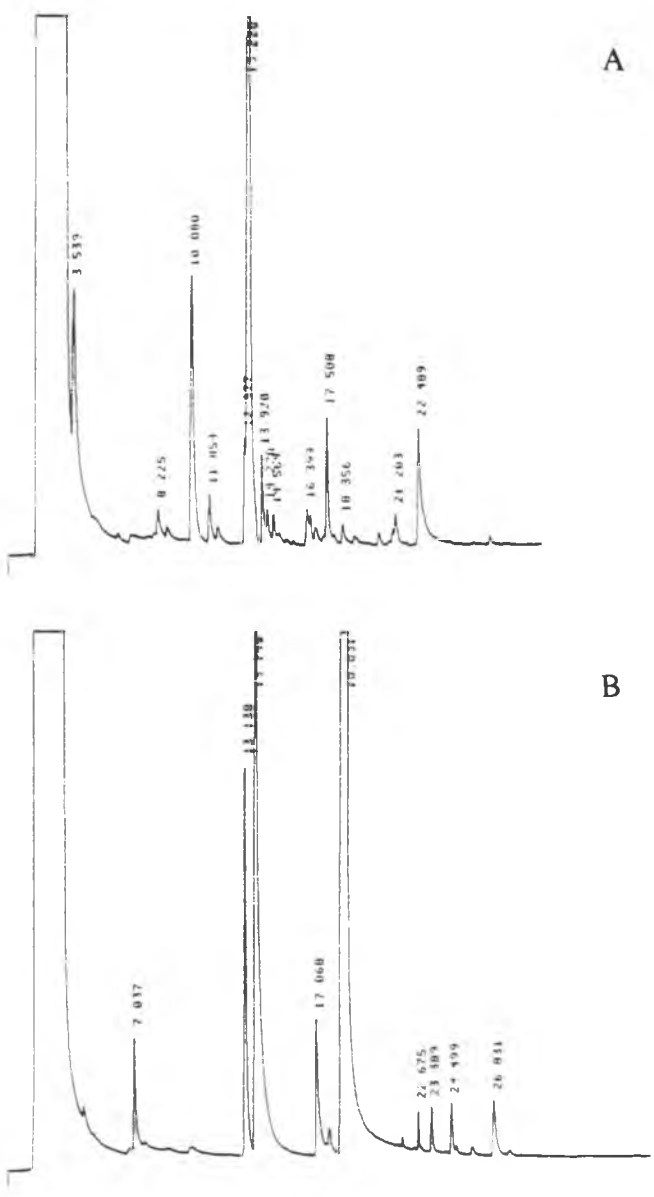

Figure 5 Chromatograms of 1213-20 samples obtained via headspace (A) and solvent extraction (B).

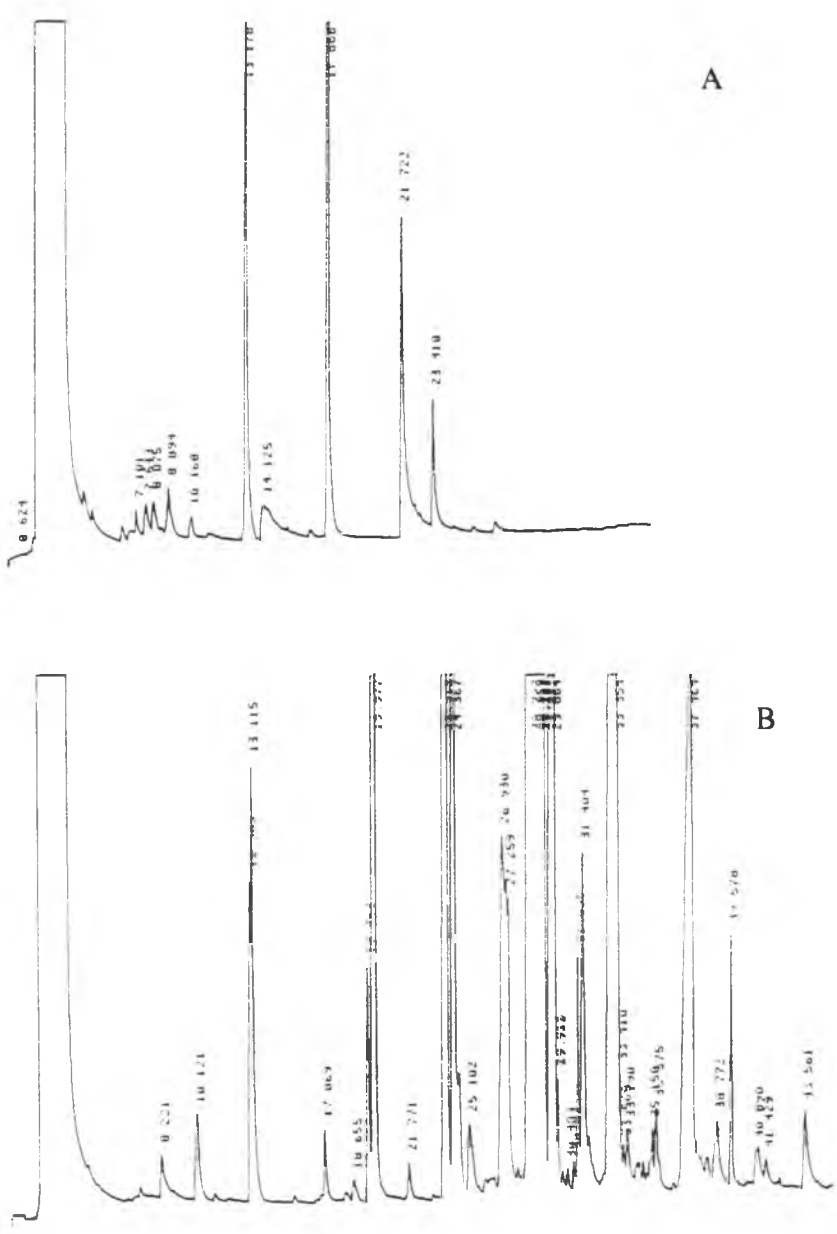

Figure 6 Chromatograms of *A.* 'Lady Beth' (A) and 1180-95 (B) samples obtained from solvent extraction.

APPENDIX 4

Table 1 Average ratings of lipid accumulation in three tepal tissues found in the base, middle and tips of fragrant and non-fragrant anthurium spadices.

Specimen	Epidermis			Sub-epidermis			Central		
	Base	Mid.	Tip	Base	Mid.	Tip	Base	Mid.	Tip
Fragrant									
A. antioquiense	2.8	4.4	2.4	4.2	6.4	5.2	3.4	4.2	5.4
A491	1.1	1.9	2.1	7.8	7.7	7.1	6.0	6.3	4.7
729	1.1	1.1	1.1	3.3	3.3	1.9	3.1	3.1	0.7
Average	1.7	2.5	1.9	5.1	5.8	4.7	4.2	4.5	3.6
Non-fragrant									
A494	2.7	2.2	2.9	4.4	5.3	0.6	4.9	4.4	1.1
UH1554	0.0	0.1	0.6	0.0	0.2	0.3	0.3	0.4	0.3
UH1750	0.7	0.4	0.0	6.2	2.3	1.0	4.7	1.8	0.6
Average	1.1	0.9	1.2	3.5	2.6	0.6	3.3	2.2	0.7

Mid = middle. A490 = *A. antioquiense*, A491 = *A. antioquiense* × *A. amnicola*, 729 = (*A.antioquiense* × *A. amnicola*) × UH757, A494 = *A. andraeanum* × *A. antioquiense*, UH1554 = UH798 × (*A. antioquiense* × 'Marian Seefurth'), UH1750 = 'Momoyama' × UH1121. Rating, from 0 to 8, with 0 cells having no lipid; 8 cells having full of lipids.

Table 2 Average ratings of starch accumulation in three tepal tissues found in the base, middle and tips of fragrant and non-fragrant anthuriums spadices.

Specimen	Epidermis			Sub-epidermal			Central		
	Base	Mid.	Tip	Base	Mid.	Tip	Base	Mid.	Tip
Fragrant									
A490	1.9	1.9	1.1	1.7	2.2	1.9	3.2	3.0	2.7
A491	1.6	2.3	1.9	3.2	2.4	2.4	2.4	2.6	2.1
729	1.2	2.3	1.6	2.2	3.2	2.2	2.0	2.3	2.1
Average	1.6	2.2	1.5	2.4	2.6	2.2	2.5	2.6	2.3
Non-fragrant									
A494	1.0	2.2	1.6	2.3	2.7	3.2	2.7	3.0	4.8
UH1554	2.7	2.4	0.2	7.1	6.8	3.3	7.3	6.3	5.6
UH1750	1.2	1.2	0.1	2.9	3.8	2.8	2.9	2.3	3.4
Average	1.6	1.9	0.6	4.1	4.4	3.1	4.3	3.9	4.6

Mid. = middle. A490 = *A. antioquiense*, A491 = *A. antioquiense* × *A. amnicola*, 729 = (*A.antioquiense* × *A. amnicola*) × UH757, A494 = *A. andraeanum* × *A. antioquiense*, UH1554 = UH798 × (*A. antioquiense* × 'Marian Seefurth'), UH1750 = 'Momoyama' × UH1121. Rating, from 0 to 8, with 0 cells having no starch; 8 cells having full of starch.

Table 3 Average ratings of lipid and starch accumulation for fragrant and non-fragrant *Anthurium*, in three different spadix zones, basal, middle and tip, and epidermis, sub-epidermal and central tepal areas.

Area	Specimens					
	basal		middle		tip	
	F	NF	F	NF	F	NF
Lipid						
Epidermal	1.67*	1.11	2.48**	0.92	1.78*	1.15
Sub-epidermal	4.74*	3.64	5.81**	2.36	4.74**	0.63
Central	4.18	3.30	4.56**	2.22	3.59**	0.67
Starch						
Epidermal	1.56	1.63	2.18	1.96	1.52**	0.63
Sub-epidermal	2.56	3.92**	2.63	3.96*	2.18	4.59**
Central	2.56	4.30**	2.63	3.89**	2.30	4.44**

F = fragrant spadix, NF = non-fragrant, * Mann-Whitney test was significant at 0.05, ** Mann-Whitney test was significant at 0.01.

Table 4 Range of lipid and starch ratings in the epidermis, sub-epidermal and central tepal areas of fragrant and non-fragrant *Anthurium*, in three different spadix zones.

Area	Specimens					
	basal		middle		tip	
	F	NF	F	NF	F	NF
Lipid						
Epidermal	1-5	0-4	1-5	0-4	0-4	0-4
Sub-epidermal	0-8	0-7	0-8	0-7	1-8	0-3
Central	0-8	0-7	1-8	0-6	0-7	0-2
Starch						
Epidermal	0-3	0-4	1-4	0-3	0-3	0-3
Sub-epidermal	1-5	1-8	1-4	1-8	1-4	1-8
Central	1-5	1-8	1-4	1-8	1-4	1-8

APPENDIX 5

Table 1 Evaluation form for scented *Anthurium* testing on CTAHR Educational Day, April 18, 1997.

Scented Anthurium Testing CTAHR, Educational day, Friday, April 18, 1997

Personal information; **Gender** _____ **F** _____**M,**

Age ___**10-20,** ___**21-30,** ___**31-40,** ___**41-50,** ___**51-60**

Please check any appropriate categories to describe anthurium scent.

Flower No.1

None	Minty	Floral	Sweet	Spicy
_____	_____	_____	_____	_____

Flower No. 2

None	Minty	Floral	Sweet	Spicy
_____	_____	_____	_____	_____

Flower No. 3

None	Minty	Floral	Sweet	Spicy
_____	_____	_____	_____	_____

Flower No. 4

None	Minty	Floral	Sweet	Spicy
_____	_____	_____	_____	_____

Flower No. 5

None	Minty	Floral	Sweet	Spicy
_____	_____	_____	_____	_____

Table 2 Summary of scented *Anthurium* testing on CTAHR Educational Fair Day, April 18, 1997.

Gender	Flower No.	Fragrance categories								
		none	minty	floral	sweet	spicy	others			
Female	1	18	18	2	4	12	1 minty & spicy	2 floral & spicy	1 minty & sweet	1 minty & sweet & spicy
	2	0	2	25	22	3	5 floral & sweet	1 minty & floral & sweet	1 minty & floral	
	3	10	9	14	21	3	1 minty & spicy	1 floral & sweet		
	4	4	26	1	4	21	1 minty & spicy	1 licorice y	1 sweet & spicy	
	5	3	4	31	12	4	4 floral & sweet	1 floral & sweet & spicy		
Male	1	18	17	2	6	16	2 minty & sweet	4 minty & spicy	1 minty & floral & spicy	1 sweet & spicy
	2	2	4	29	24	2	5 floral & sweet	1 minty & sweet		
	3	19	7	15	13	6	3 floral & sweet	2 floral & spicy	1 minty & floral	1 sweet & spicy
	4	4	23	5	5	26	1 minty & floral	3 minty & spicy		
	5	5	6	26	17	4	8 floral & sweet	1 minty & sweet		

1 = *A.* 'ARCS' (3 flowers), 2 = *A.* 'Lady Beth' (3 flowers), 3. UH1299 (2 flowers), 4 = 633-41 (3 flowers) and 5 = 649-7 (2 flowers).

APPENDIX 6

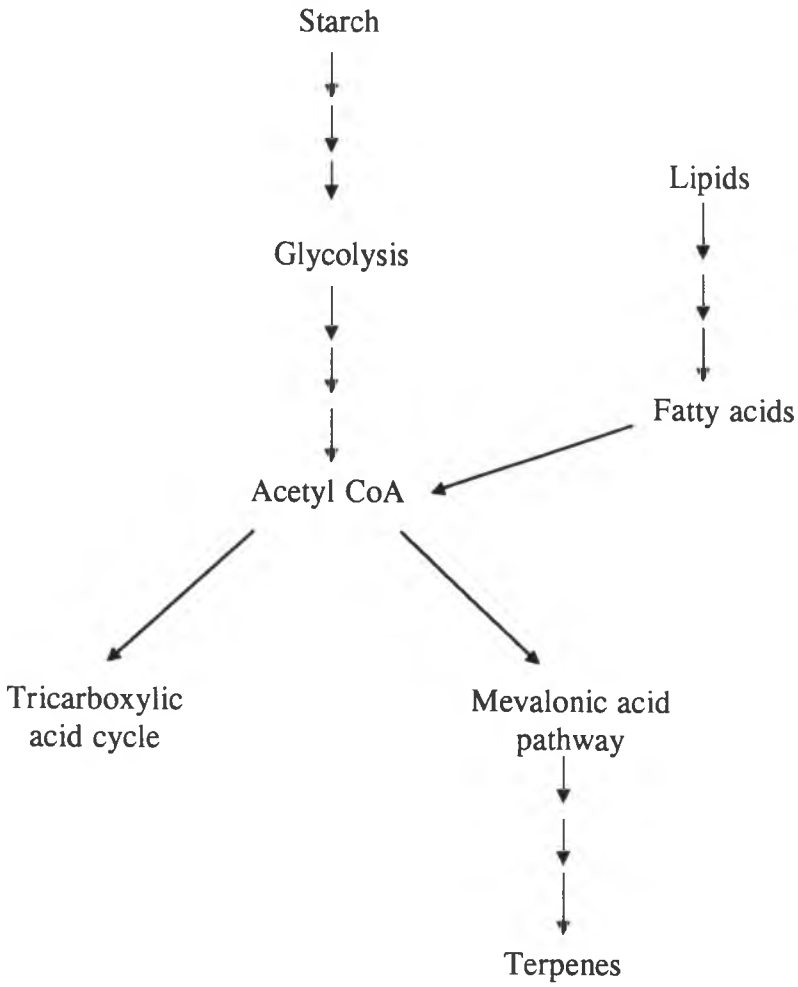

Figure 1 Overview of terpene synthesis, from acetyl CoA via the mevalonic acid pathway.

APPENDIX 7

List of terms and definitions provided to the expert panel.

aldehydic	a floral bouquet harmonized with a complex of fatty aldehydes which contribute to the fragrance blend.
animal	remisniscent of either musk Tonkin, castoreum, civet or ambergris and contains a warm, vibrant nuance.
balsamic	a mild sweet, vanillic note with a slighty woody background.
citrus	reminiscent of citrus fruits.
dry	describes the absence of sweetness.
floral	refers to a particular flower and at other times refers to the combined fragrances of several flowers.
fresh	refers to the green note of recently cut leaves, the clean and invigorating scent of early morning air, or an ozonic smell.
fruity	not for citrus; suggestive of any of the edible fruits.
green	freshly cut leaves or vines, or fresh, leafy scent.
herbaceous	a medicinal or phenolic note.
honey	sweet, heavy and syrupy with a waxy background.
lavender	
mint	
mossy	suggestive of the aromatic lichens and mosses, particularly oakmoss and tree moss.

resinous	refers to gum exuded from trees.
smoky	the burning of odoriferous woods resulting in sweet-smelling smoke.
spicy*	shared common pungency and sharpness that stimulate the senses of smell and taste.
sweet	a rich fragrance with the ambrosial characteristics of a sweet taste
vanilla	
woody	suggestive of aromatic woods and roots.

* The expert panel defined "spicy" as reminiscent of spices.

Reference

Thiboud, M. 1991. Empirical classification of odours. (*In*) P. M. Muller and D. Lamparsky, eds. *Perfumes: art, science and technology*. Elsevier Applied Science, New York. pp. 253-286.

REFERENCES

Ackerman, D. 1991. *A natural history of the senses*. Vintage Books, A Division of Random House, Inc., New York. 331 pp.

Allen, E. F. 1980. Rose scent- A quantitative study. *The Rose Ann*. pp. 58-61.

Altenburger, R. and P. Matile. 1988. Circadian rhythmicity of fragrance emission in flowers of *Hoya carnosa* R. Br. *Planta* 174:248-252.

Altenburger, R. and P. Matile. 1990. Further observation on rhythmic emission of fragrance in flowers. *Planta* 180:194-197.

Anderton, E. and R. Park. 1989. *Growing gladiolus*. Christopher Helm, London. 166 pp.

Baker, J. R. 1958. *Principles of biological microtechnique*. John Wiley & Sons Inc., New York. 375 pp.

Barletta, A. 1995. Scent makes a come back. *FloraCul. Inter*. pp. 23-25.

Baumgardt, J. P. 1982. *How to identify flowering plant families*. Timber Press, Portland, Oregon. 269 pp.

Bermadinger-Stabentheiner, E. and A. Stabentheiner. 1995. Dynamics of thermo-genesis and structure of epidermal tissues in inflorescences of *Arum maculatum*. *New Phytol*. 131:41-50

Bouquet, A. 1968. Perfumes of the rose. *Amer. Rose Ann*. 53: 107-111.

Bown, D. 1988. *Aroids*. Timber Press, Portland, Oregon. 256 pp.

Boyce, P. 1995. Introduction of the Family Araceae. *Curtis's Botanical Magazine* 12: 122-125.

Breer, H. 1997. Sense of smell: signal recognition and transduction in olfactory receptor neurons. (In) E. Kress-Rogers, ed. *Handbook of biosensors and electronic noses: medicine, food and environment*. CRC Press, Inc., New York. pp.521-532.

Bronner, R. 1975. Simultaneous demostration of lipids and starch in plant tissue. *Stain Tech*. 50:1-4.

Brunke, E. J., F. J. Hammerschmidt and G. Schmaus. 1992. The headspace analysis of flower fragrances. *DRAGOCO Rep*. pp. 3-31.

Buchanan, R. 1995. Taylor's guide to herbs. Houghton Mifflin Co., New York. 453 pp.

Burbott, A. J. and W. D. Loomis. 1967. Effects of light and temperature on the monoterpenes of peppermint. *Plant Physiol*. 42:20-28.

Calkin, R. R. and J. S. Jellinek. 1994. *Perfumery: Practice and principles*. John Wiley & Sons, Inc., New York. 287 pp.

Carruth, T. 1992. The fickle finger of fragrance. *Amer. Rose Ann*. 77:24-25.

Civille, G. V. and J. Close. 1994. Fragrance spectrum descriptive analysis workshop, September 12-14. New Jersey.

Croat, T. B. 1980. Flowering behavior of the neotropical genus *Anthurium* (Araceae). *Amer. J. Bot*. 67: 888-904.

Croat, T. B. 1992. Species diversity of Araceae in Colombia: A preliminary survey. *Ann. Missouri Bot. Gard*. 79:17-28.

Croteau, R. and F. Karp. 1991. Origin of natural odorants. (*In*) P. M. Muller and D. Lamparsky, eds. *Origin of natural odorants*. Elsevier Applied Science, New York. pp.101-126.

Curry, K. J. 1987. Initiation of terpenoid synthesis in osmophores of *Stanhopea anfracta* (Orchidaceae): a cytochemical study. *Amer. J. Bot.* 74:1332-1338.

Dafni, A. 1992. *Pollination Ecology*. IRL Press, New York. 250 pp.

Dahlgren, R. M. T., H. T. Clifford and P. F. Yeo. 1985. *The families of the monocotyledones structure, evolution and taxonomy*. Springer-Verlag, New York. 354 pp.

Dhulappanavar, C. V. 1976. Inheritance of scent in rice. Euphytica 25:659-662.

DLO. 1997. Fragrance research. *FloraCul. Inter.* p.32.

Dobson, H. E. M. 1991. Analysis of flower and pollen volatiles. (*In*) H. F. Linskens and J. F. Jackson, eds. *Modern methods of plant analysis*. Springer-Verlag, Berlin, Germany. pp. 231-251.

Dudareva, N., L. Cseke, V. M. Blance and E. Pichersky. 1996. Evolution of floral scent in *Clarkia*: novel patterns of *s*-linalool synthase gene expression in the *C. breweri* flower. *Plant Cell* 8: 1137-1148.

Esau, K. 1977. *Anatomy of seed plants*. John Wiley & Sons, Inc., New York. 550 pp.

Feder, N. and T. P. O'Brien. 1968. Plant microtechnique: some principles and new methods. *Amer. J. Bot.* 55:123-142.

Forrest, G. W. 1945. Foreward to fragrance. *Amer. Rose Ann.* 30: 66-70.

Fowlis, I. A. 1995. *Gas chromatography*, 2nd ed. John Wiley & Sons Ltd., Chichester, England. 258 pp.

Franz, C. 1993. Genetics. (*In*) R. K. M. Hay and K. P. Svoboda, eds. *Volatile oil crops: their biology, biochemistry and production*. Longman Scientific & Technical, England. pp. 63-96.

Geddes, A. J. 1980. *Mass spectrometry. (In)* S . B. Brown, ed. *An introduction to spectroscopy for biochemists*. Academic Press, New York. pp. 336-392.

Gerlach, G. and R. Schill. 1989. Fragrance analyses, an aid to taxonomic relationships of the genus *Coryanthes* (Orchidaceae). *Pl. Syst. Evol.* 168:159-165.

Gershenzon, J. and R. Croteau. 1990. Regulation of monoterpene biosynthesis in higher plants. *Recent Adv. Phytochem.* 24:99-160.

Gibbsons, B. 1986. The intimate sense of smell. *Nat. Geo.*, 170: 324-360.

Grayer, R. J., G. C. Kite, F. J. Goldstone, S. E. Bryan, A. Paton and E. Putievsky. 1996. Infraspecific taxonomy and essential oil chemotypes in sweet basil, *Ocimum basilicum*. *Phytochem.* 43:1033-1039.

Gupta, S. C. 1994. Genetic analysis of some chemotypes in *Ocimum basilicum* var *glabratum*. *Plant Breeding* 112: 135-140.

Harborne, J. B. and B. L. Turner. 1984. *Plant chemosystematics*. Academic Press, Orlando, Florida. 562 pp.

Harkness, P. 1992. Roses for noses. *Amer. Rose* pp. 15-17.

Hawaii Agriculture Statistics Service. 1997. *Hawaii flowers & nursery products; annual summary*. Hawaii Department of Agriculture, U. S. Department of Agriculture, June 27, 20 pp.

Hay, R. K. M. and K. P. Svoboda. 1993. Botany. (*In*) R. K. M. Hay and P. G. Waterman, eds. *Volatile oil crops: their biology, biochemistry and production*. Longman Scientific & Technical, England. pp. 5-22.

Heywood, V. H. 1985. *Flowering plants of the world*. Croom Helm Publishers Ltd., Yugoslavia. 335 pp.

Higaki, T., H. P. Rasmussen and W. J. Carpenter. 1984. A study of some morphological and anatomical aspects of *Anthurium andraeanum* Lind. College of Tropical Agriculture and Human Resources, University of Hawaii. Research Series 030.

International Floriculture Quarterly Report. 1996. *Cut flower sales through the Dutch auctions 1993-1995*. Pathfast Publishing, Essex. 5:12-15.

Jasik, E. R. 1993. Fragrant roses and Henri Delbard. *The Amer. Rose* 32: 12-13.

Jodon, N. E. 1944. The inheritance of flower fragrance and other characters in rice. *J. Amer. Soc. Agron.* 36: 844-848.

Kamemoto, H., R. Y. Iwata and M. Marutani. 1988. Genetics of the major spathe colors in Anthurium. College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Research Extension Series 056.

Kamemoto, H. and A. R. Kuehnle. 1996. *Breeding anthuriums in Hawaii*. University of Hawaii Press, Honolulu, Hawaii. 132 pp.

Kite, G. C. 1995. The floral odour of Arum maculatum. Biochem. Sys. Ecol. 32: 343-354.

Knudsen, J. T. and L. Tollsten. 1991. Floral scent and intrafloral scent differentiation in *Moneses* and *Pyrola* (Pyrolaceae). *Plant Syst. Evol.* 177: 81-91.

Knudsen, J. T., L. Tollsten and L. G. Bergstrom. 1993. Floral scents- a checklist of volatile compounds isolated by head-space techniques. *Phytochem.* 33: 253-280.

Knutson, R. M. 1974. Heat production and temperature regulation in eastern skunk cabbage. *Science* 186:746-747.

Kuanprasert, N., A. R. Kuehnle and C. S. Tang. 1997 (in submission).

Kuehnle, A. R. and F. R. Chen. 1994. Genetic transformation in *Anthurium*. (*In*) Y. P. S. Bajaj, ed. *Biotechnology in agriculture and forestry*. Springer-Verlag, Berlin. pp. 217-225.

Kuehnle, A., J. Halloran, H. Kamemoto, J. Lichty, T. Amore and N. Sugii. 1996. Anthurium cut flower breeding and economics. College of Tropical Agriculture & Human Resources, University of Hawaii at Manoa, Research Extension Series 165.

Matsumoto, T. K., A. R. Kuehnle and D. T. Webb. 1995. Improvements for observing *Anthurium* (Araceae) floral structures by light microscopy. *Biotech. & Histochem.* 70:205-210.

McLean, F. T. 1933. The inheritance of fragrance in gladiolus species crosses. *The New York Bot. Gard.* 34: 73-80.

McLean, F. T. 1938. A genetic analysis of the inheritance of fragrance in gladiolus. *Bull. Torr. Bot. Club* 65:181-197.

Meeuse, B. and S. Morris. 1984. *The sex life of flowers*. Facts on File Publications, New York. 152 pp.

Meilgaard, M., G. V. Civille and B. T. Carr. 1991. *Sensory evaluation techniques*. CRC Press, Inc., Boca Raton, Florida. 281 pp.

Merriam-Webster. 1991. *Webster's ninth new collegiate dictionary*. Merriam-Webster Inc., Springfield, Massachusetts. 1563 pp.

Metcalf, R. L. 1987. Plant volatiles as insect attractants. *CRC Crit. Rev. Plant Sci.* 5: 251-301.

Mookherjee, B. D., R. W. Trenkle and R. A. Wilson. 1990. The chemistry of flowers, fruits and spices: live vs. dead a new dimension in fragrance research. *Pure & Appl. Chem.* 62:1357-1364.

Morris, E. T. 1984. *Fragrance*. Charles Scribner's Sons, New York. 304 pp.

Nagaraju, M., D. Chaudhary and M. J. B. Rao. 1975. A simple technique to identify scent in rice and inheritance pattern of scent. *Cur. Sci.* 44:599.

Neuner-Jehle, N. and F. Etzweiler. 1991. The measuring of odors. (*In.*) P. M. Muller, and D. Lamparsky, eds. *Perfumes: arts, science and technology*. Elsevier Applied Science, New York. pp. 153-212.

Nishizawa, A., G. Honda and M. Tabata. 1992. Genetic control of the enzymatic formation of cyclic monoterpenoids in *Perilla frutescens*. *Phytochem.* 31: 139-142.

Northen, R. T. 1990. *Home orchid growing*. Simon & Schuster, New York. 376 pp.

Ody, P. 1993. *The complete medicinal herbal*. Dorling Kindersley, New York. 192 pp.

Omata, A., S. Kakamura, K. Yomogida, K. Moriai, Y. Ichikawa and I. Watanabe. 1990. Volatile compounds of TO-YO-RAN flowers (*Cymbidium faberi* and *Cymbidium virescens*). *Agri. Biol. Chem.* 54:1029-1033.

Omata, A., K. Yomogida, Y. Teshima and S. Nakamura. 1991. Volatile components of ginger flowers (*Hedychium coronarium* Koenig). *Fla. Frag. J.* 6:217-220.

Pinson, S. R. M. 1994. Inheritance of aroma in six rice cultivars.*Crop Sci.* 34: 1151-1157.

Pridgeon, A. M. and W. L. Stern. 1983. Ultrastructure of osmophores in *Restrepia* (Orchidaceae). Amer. J. Bot. 70:1233-1243.

Proctor, M., P. Yeo and A. Lack. 1996. *The natural history of pollination*. Timber Press, Portland, Oregon. 479 pp.

Robacker, D. C., B. J. D. Meeuse and E. H. Erickson. 1988. Floral aroma. *BioSci.* 38: 390-398.

Roundnistka, E. 1991. The art of perfumery. (*In*) P. M. Muller and D. Lamparsky, eds. *Perfumes: arts, science and technology*. Elsevier Applied Science, New York. p. 3-48.

Simmond, N. W. 1987. *Principles of crop improvement*. 4th ed. Longman Scientific & Technical, London. 408 pp.

Sivarajan, V. V. 1991. Sources of taxonomic characters. (*In*) N. K. P. Robson, ed. *Introduction to the principles of plant taxonomy*. Cambridge University Press, Melborne, Australia pp. 170-197.

Skubatz, H., D. D. Kunkel and B. J. D. Meeuse. 1993. Ultrastructural changes in the appendix of the *Sauromatum guttatum* inflorescence during anthesis. *Sex Plant Reprod.* 6:153-170.

Skubatz, H. and B. J. D. Meeuse. 1993. Energy loss in tissue slices of the inflorescence of *Sauromatum guttatum* (Schott) analysed by microcalorimetry. *J. Exp. Bot.* 44:493-499.

Skubatz, H., D. D. Kunkel, J. M. Patt, W. N. Howald, T. G. Hartman and B. D. Meeuse. 1995. Oleic acid and its positional isomer, cis-vaccine acid, in the appendix of *Sauromatum guttatum* during anthesis. *Proc. Nat. Acad. Sci.* 92:10084-10088.

Smith, B. N. and B. J. D. Meeuse. 1966. Production of volatile amines and skatole at anthesis in some arum lily species. *Plant Physiol.* 41:343-347.

Sprent, P. 1993. *Applied nonparametric statistical methods*. Chapman & Hall, Suffolk. 342 pp.

Srb, A. M., R. D. Owen and R. S. Edgar. 1965. *General genetics*. W. H. Freeman and Company, San Francisco. 557 pp.

Surburgh, H., M. Guentert and H. Harder. 1993. Volatile compounds from flowers: Analytical and olfactory aspects. (*In*) R. Teranishi, R. G. Buttery and H.

Sugisawa, eds. *Bioactive volatile compounds from plants*. American Chemical Society, Washington DC. pp. 168-186.

Taiz, L. and E. Zeiger. 1991. *Plant physiology*. The Benjamin/Cummings Publishing Company, Inc., Redwood City, California. 559 pp.

Thiboud, M. 1991. Empirical classification of odours. (*In*) P. M. Muller and D. Lamparsky, eds. *Perfumes: arts, science and technology*. Elsevier Applied Science, New York. p. 253-286.

Tingey, D. T., M. Manning, L. C. Grothaus and W. F. Burns. 1979. The influence of light and temperature on isoprene emission rates from live oak. *Physiol. Plant* 47:112-118.

Tollsten, L., J. T. Knudsen and L. G. Bergstrom. 1994. Floral scent in generalistic Angelica (Apiaceae)- an adaptive character? Biochem. Syst. Ecol. 22:161-169.

Tucker, A. O., H. Hendrik, R. Bos and D. E. Faribrothers. 1991. The origin of *Mentha x gracilis* (Lamiaceae). II. Essential oils. *Econ. Bot.* 45: 200-215.

Vogel, S. 1990. *The role of scent glands in pollination*. A. A. Balkema, Rotterdam. 202 pp.

Wannakrairoj, S. and H. Kamemoto.1990. Inheritance of purple spathe in *Anthurium*. *J. Amer. Soc. Hort. Sci.* 115: 169-171.

Whitten, W. M., H. G. Hills and N. H. Williams. 1988. Occurrence of ipsdienol in floral fragrances. *Phytochem.* 27:2759-2760.