Diversity of fungi on wild fruits in Hong Kong

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Eighteen species of wild fruits were examined for fruit-decaying fungi in nature. Surface-sterilized and non-sterilized fruits were incubated for 1-4 weeks and the fruiting bodies were identified. A total of 540 samples and 495 microfungi from 102 taxa were identified. *Colletotrichum* and *Phomopsis* were the most frequently recorded fungal genera. *Ilex cinerea* had the most diverse fungal species, while *Wikstroemia nutans* had the lowest diversity. Fifty-eight percent of fungal genera found in this survey have not been recorded on cultivated fruits. The fungal community that developed on non-sterilized fruits after incubation was generally more diverse than on surface-sterilized fruits, but both were colonized mostly by non-specific fungi. Related fruit species did not, in general, have more similar fungal communities than unrelated species. The ability of detached fruits to resist fungal infection under incubation varied greatly, with 77% of fruits of *Wikstroemia nutans* still not infected after 4 weeks. Fungi were classified into three types: pathogens, latent opportunists and fast-colonizing opportunists. Fast-colonizing opportunists, such as *Cladosporium*, *Fusarium* and *Penicillium*, were the most important taxa on wild fruits.

Keywords: fruit fungi, microfungi, wild fruits

Introduction

Different fungal assemblages are present on almost every plant part of a single host (Hyde, 1995). They may be present as phylloplane fungi, saprobes, endophytes, mycorrhizal fungi, parasites and commensals. Their roles in nature are not always clearly defined and the reasons for their widespread occurrence may be unknown (Parbery, 1996). Fungi associated with fleshy fruits are one of the less well-defined assemblages. Fruits are different from other plant parts because they have evolved for seed dispersal. The highly nutritious nature of the fruit itself represents an attractive reward not only to their dispersers, but also to microbes. This conflicting relationship between fruits, dispersers and fruit-rotting agents is of considerable interest because plants may have

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developed the ability to retain ripe fruits on plants for a long time to enhance dispersal. This ability requires physical and chemical defense against microbes. Because of the probable unattractive nature of the defensive chemicals to dispersers, the occurrence of defensive compounds within ripe pulp represents a tradeoff with respect to defense from damaging agents and palatability for dispersers (Herrera, 2002).

Because of this tradeoff between defense and palatability, defensive chemicals in and on ripe fruits cannot provide complete protection. Whenever there is a virulent pathogen colonizing the substrate, the chance of infection of fruits is high. In addition, the concentration of defense chemicals generally declines during ripening, the fruits then become more vulnerable to pathogens (Herrera, 2002).

There have been no comprehensive surveys of the diseases of wild fruits in the tropics or elsewhere. Previous studies only focused on diseases of commercial fruits, with emphasis on postharvest diseases (Wun, 1991; Zhang *et al.*, 1998; Tang, 2000) or seeds (Somrithipol *et al.*, 2002). Commercial fruits, due to centuries of genetic modification, are different from wild fruits in terms of size, chemical defenses and nutritional content. Postharvest diseases develop during transport and storage, which have no equivalent in nature. Therefore, a study was initiated to find out what kinds of fungi are responsible for fruit decay in nature.

Materials and methods

The study area

Hong Kong is situated on the southern coast of China (Latitude 22°9′N-22°37′N, longitude 113°52′E-114°30′E) and has a subtropical monsoonal climate with highly seasonal rainfall and temperature. A hot, wet, monsoon season dominates from early May to the end of September, including 70% of annual total rainfall; it is replaced by a cool, dry season from November to March. The seasons are generally separated by two shorter periods of transitional weather (Dudgeon and Corlett, 1994). The mean monthly temperature ranges from 28.8°C in July to 15.8°C in January. However, during the study period of the survey (January 2001-August 2002), the temperature was warmer than usual. The mean temperatures ranged from 29.2°C for August 2001 to 17.3°C in January 2002.

Despite centuries of deforestation due to human impact, Hong Kong still has a surprisingly rich flora (Corlett and Turner, 1997). It is estimated that there are approximately 1800 species of native angiosperms, of which at least 482 species (27%) bear fleshy, presumably vertebrate-dispersed fruits, including 76% of the 337 tree and shrub species and 70% of the 103 climber species (Corlett, 1996). Fruits in Hong Kong have a highly seasonal fruiting phenology, with fruit availability and diversity reaching a maximum in December and January, coinciding with the peak density of migrant birds, many of which are at least partly frugivorous (Corlett, 1998).

Collection, processing and examination of samples

Collections were made at Lung Fu Shan, Hok Tau, Mount Nicholson, Pat Sin Leng, Tai Po Kau, Hong Kong, from September 2001 to June 2002. Wild fruits were collected from 18 native plant species in Hong Kong, representing twelve angiosperm families (Table 1). Three species of *Ilex* and *Ardisia* were chosen to compare the similarities of fungi on them. All of them are common plants in Hong Kong, inhabiting shrubland, forest, or grassland. They are all at least partly dispersed by birds in Hong Kong, and fruit at different times of the year.

Six mature fruits each were collected haphazardly from five individual plants in the field and placed in separate "zip-lock" plastic bags. This sample size was chosen because some plant species only have a few fruits per plant. All the samples were returned to the laboratory and half were subjected to surface sterilization (see below). They were then incubated at room temperature (~23^{o}C) in plastic bags with sterilized moist tissue. From an initial trial, a period of 1-3 weeks was found to be the optimum time for the development of fruiting bodies on fruits.

The technique chosen in this study was similar to that used for the study of endophytic fungi of other plant parts. The aim is to remove all epiphytic fungi from the exterior of the fruit skin and encourage the growth of the internal mycota (Taylor *et al*., 1999; Fröhlich *et al*., 2000). The samples were dipped in 95% ethanol for 1 minute, followed by 10 minutes in 3.25% sodium hypochlorite (using Chlorox® diluted with distilled water at a ratio of 6:4) and finally, 30 seconds in 95% ethanol.

The samples were examined to identify fungal structures under a stereo-microscope. Squash mounts and sections of fungal fruit bodies were mounted in water for measurement and photographed with a digital camera. Fungi were isolated and are maintained in The University of Hong Kong Culture Collection (HKUCC) and fruits with fungi were dried and deposited in The University of Hong Kong Herbarium (HKU(M)).

Data analysis

The numbers of fungal species were recorded and fungal diversity of different species of wild fruits was evaluated by Shannon's Diversity Index (Begon *et al*., 1993). The species composition between different wild fruit species was compared by cluster analysis (Magurran, 1988). An agglomerative hierarchical method was used to produce a cluster dendrogram, which was generated from a software package PC-ORD version 4 (McCune and Mefford, 1999). Euclidean distance was chosen as the cluster distance measure and the Ward's method (error sum of squares) was chosen as a general purpose linkage method that minimizes distortions in the underlying space. The relationship between the number of fungal species and mean surface area (calculated from mean diameter) was investigated by linear regression.

Table 1. Families and species of the fruits in this study

Family	Fruit species	Site	Fruit season	Colour
Aquifoliaceae	*Ilex asprella*	Lung Fu Shan	May-June	Purple
	Ilex cinerea	Lung Fu Shan	Nov-Dec	Red
	Ilex pubescens	Lung Fu Shan	Dec-Jan	Red
Araceae	*Alocasia odora*	Lung Fu Shan	May-June	Red
Caprifoliaceae	*Viburnum sempervirens*	Pat Sin Leng	Nov-Dec	Red
Chloranthaceae	*Sarcandra glabra*	Tai Po Kau	Dec-Jan	Orange
Euphorbiaceae	*Bridelia tomentosa*	Lung Fu Shan	Jan-Feb	Black
Hydrangeaceae	*Dichroa febrifuga*	Tai Po Kau	Jan	Blue
Lauraceae	*Litsea rotundifolia*	Lung Fu Shan	Oct-Nov	Black
Myrsinaceae	*Ardisia crenata*	Lung Fu Shan	Dec-Feb	Red
	Ardisia punctata	Victoria Peak	Dec-Feb	Red
	Ardisia quinquegona	Tai Po Kau	Nov-Dec	Black
Myrtaceae	*Cleistocalyx operculatus*	Hok Tau	Sep-Oct	Red
	Rhodomyrtus tomentosa	Pat Sin Leng	Oct-Nov	Red
Rubiaceae	*Diplospora dubia*	Victoria Peak	Nov-Dec	Red
	Psychotria asiatica	Lung Fu Shan	Nov-Jan	Red
Tiliaceae	*Microcos paniculata*	Hok Tau	Oct-Nov	Black
Thymelaeaceae	*Wikstroemia nutans*	Lung Fu Shan	April-May	Orange

Results and discussion

Overview of fungi on wild fleshy fruits

The results of this study are preliminary observations on the fungi associated with wild fruits in Hong Kong. The aim was to identify the kind of fungi responsible for fruit decay in nature with respect to the conflicting relationship between fruits, dispersers and fruit-rotting agents. Due to the availability of the wild fruits in the field, the sample size was relatively small for particular species. The results however, represent the first data on the diversity of fungi on wild fleshy fruits.

A total of 540 samples from 18 wild fruit species were examined for fungi, and 495 fungi from 101 taxa of microfungi were identified (Table 2). These comprised 80 (79%) species of anamorphic fungi—56 (54%) coelomycetes and 25 (25%) hyphomycetes—18 (18%) ascomycetes, 2 (2%) myxomycetes and 1 (1%) oomycete. Almost three-quarters of the genera collected in this survey were represented by a single species and 31% of the species recorded were collected only once. About one-third (39%) of genera occurred on 3 or more fruit hosts. The most widespread fungal genera in terms of host occurrence and number of collections were *Colletotrichum* and *Phomopsis*, which were found on 16 and 15 fruit hosts studied, and accounted for 76 and 67 collections respectively.

Eleven genera from nine ascomycete families were recorded in the present study. All families were represented by a single genus, except *Botryosphaeriaceae* and *Lophiostomataceae* with two genera. The most common genera were *Guignardia, Glomerella* and *Massarina*, the former being found on seven and the latter two on four fruit hosts. The most frequently collected species was *Guignardia cocogena* (11 collections). One ascomycete was found to be a new species in the family *Lophiostomataceae*. It was named *Lophiotrema psychotrii* sp. nov. (Tang *et al*., 2003).

Forty genera of anamorphic fungi were identified. These comprised 23 genera of coelomycetes and 17 genera of hyphomycetes. Seventy-three percent of the genera were represented by single species. The most common genera in terms of species, total collections and host occurrence were *Phoma* (10 species, 23 collections, 11 hosts), *Pestalotiopsis* (9 species, 18 collections, 8 hosts), *Phomopsis* (5 species, 67 collection, 15 hosts) and *Colletotrichum* (5 species, 76 collections, 16 hosts). Other common genera were *Penicillium* (6, 36, 8), *Fusarium* (3, 26, 10) and *Cladosporium* (1, 41, 10). The most frequently collected species were *Colletotrichum gloeosporioides* (48 collections), *Cladosporium cladosporioides* (41 collections), *Phomopsis archeri* (30), *Phomopsis stipata* (27) and *Colletotrichum musae* (23).

Conidioxyphium sp. was found associated with a scale insect, the Cockerell Scale, *Pseudaulacaspis cockerelli* Cooley (Homoptera: Coccoidea: Diaspididae) in November-December 2001 on *Psychotria asiatica* fruits and stems (Tang, 2002). On plants colonized by this scale insect, *Conidioxyphium* sp. colonized the whole plant and blighted shoots and fruit rots were observed.

The taxonomic distribution of fungal classes was quite consistent on different fruit hosts, with anamorphic fungi as the dominant fungal class on all 18 hosts. The average ratio of anamorphic to ascomycete taxa in 18 species of wild fruits was 10.5:1. The dominance of anamorphic fungi varied from 67% on *Microcos paniculata* to 100% on *Wikstroemia nutans*. Some of the hosts, including *Ardisia quinquegona*, *Bridelia tomentosa*, *Cleistocalyx operculatus*, *Ilex cinerea* and *Wikstroemia nutans*, had no abundance of ascomycetes, while *Dichroa febrifuga* and *Viburnum sempervirens* had the highest percentages of ascomycetes (27%).

The wide occurrence of the fungal genera *Colletotrichum* and *Phomopsis* confirms that they are extremely prevalent genera on both native and cultivated fruits (Salunkhe and Desai, 1984). Shivas and Hyde (1997) suggested that these two genera are extremely diverse as saprobes, pathogens and endophytes, and able to infect fruits either early in their development as quiescent infection, or opportunistically colonize wounds and lesions made by other pathogens. *Colletotrichum* is a major pathogen genus with a worldwide distribution recorded on more than 470 different hosts (Waller, 1992). There are 39 accepted species (Sutton, 1992) with some species capable of producing an array of pectolytic enzymes (Prusky *et al*., 1989). Prusky *et al*. (2001) has shown that three species, *Colletotrichum gloeosporioides*, *C. acutatum* and *C. coccodes*, are able to secrete ammonia locally in their host, resulting in a pH increase that enables secretion of pectate lyase and enhanced virulence. *Phomopsis* is a destructive genus which is known to attack many vegetables and fruits. At least 60 species of *Phomopsis* are known to be pathogenic on plants (Wehmeyer, 1933) and many pectolytic enzymes were recently characterized to explain the reasons for success of this pathogen (Bruton *et al*., 1998; Zhang *et al*., 1999). *Phomopsis cucurbitae* can produce at least eight polygalacturonases isozymes to utilize various substrates in different environments leading to an increased degree of virulence (Zhang *et al*., 1999).

The wide occurrence of other fungal genera, including *Cladosporium*, *Fusarium*, and *Penicillium,* suggests that they are ubiquitous in air and are able to rapidly infect wounded and overripe fruits. They are characterized by rapid colonization of substrate and production of large amounts of light conidia.

It is worth noting the collection of two genera of myxomycetes, *Physarum* and *Stemonitis*. The occurrence of slime molds on mature fruit is not common in nature, because they normally grow on decaying wood, bark or leaf litter and in restricted environmental conditions as they engulf bacteria. The environment for the growth of slime molds must be very moist and under shade of the tree canopy. *Physarum nutans* was found in Tai Po Kau Nature Reserve (the largest contiguous forest area in Hong Kong) on *Dichroa febrifuga,* which is an understorey shrub under deep shade of *Machilus* trees (14-18 m tall).

Species diversity and abundance of fungi on wild fruits

The highest species diversity was recorded on *Ilex cinerea* fruits (H = 2.82), followed by *Psychotria asiatica* (H = 2.759) and *Viburnum sempervirens* (H = 2.59), and the lowest diversity on *Wikstroemia nutans* (H = 0.74) (Table 3). *Psychotria asiatica* and *Ilex cinerea* also had the highest number of fungal species collected (19).

Most (72%) non-sterilized fruits had more fungal species and higher species diversity than sterilized ones (Table 4). The number of species collected on sterilized fruits ranged from two on *Bridelia tomentosa* and *Wikstroemia nutans* to nine on *Dichroa febrifuga*, while the number on non-sterilized fruits ranged from two on *Dichroa febrifuga* to 16 on *Psychotria asiatica*. The highest fungal diversity on sterilized fruits was recorded on *Ilex pubescens* (H = 2.03), followed by *Viburnum sempervirens* (H = 2.02) and *Dichroa febrifuga* (H = 1.95), whereas the lowest was found on *Ardisia punctata* (H = 0.45). The highest diversity on non-sterilized fruits was recorded on *Psychotria asiatica* (H = 2.73), followed by *Ilex cinerea* (H = 2.52) and *Viburnum sempervirens* (H = 2.34), while the lowest value was found on *Dichroa febrifuga* (H = 0.69).

The relationship between the number of fungal species and mean surface area was not significant. This suggests that other factors such as nutrients, antimicrobial compounds and the physical structure are more important. For example, Domergue *et al*. (2000) showed that alkaloids and terpenoids in the idioblast cells of Avocado inhibit *in vitro* growth of fungi. Thomas *et al*. (1992) also demonstrated that variation in the fine physical structure of substrates could affect penetration and colonization of fungi.

Approximately 20% of the total fruits examined were found to have no observable fungal infections on the fruit surface after 4 weeks of incubation. This was particularly obvious for surface sterilized fruits (25%) than non-sterilized ones (17%). The highest proportion of fruits with no fungi was found in *Wikstroemia nutans* (77%). *Wikstroemia nutans* also had the highest proportion of sterilized fruits with no fungi (80%), whereas *Dichroa febrifuga* had the highest proportion of non-sterilized fruits (87%).

The higher fungal diversities of non-sterilized fruits than sterilized ones and the higher proportion of sterilized fruits with no observable fungal infections after one month of incubation suggests that surface microbes are more diverse than endophytes in fruits. It is likely that wild fruits are decayed mostly by surface microbes, while the growth of endophytes are generally inhibited before the senescence of the fruits. This is different from cultivated fruits which are more likely to be decayed by both surface microbes and "re-activated" endophytes during ripening period (Johnson *et al*., 1992; Duggan and Roberts, 1994; Oudemans *et al*., 1998).

Similar species of fungi were recovered from different species of sterilized fruits. *Botryodiplodia*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Phoma*, *Phomopsis*, *Pestalotiopsis* species and many species of sterile mycelia were found in many fruit species. Among them, *Phomopsis* and *Colletotrichum* were the most abundant genera, accounting for 23% and 19% of the fungi on sterilized fruits respectively, and both on 15 fruit hosts. These species are suspected to be endophytes. They are similar to fungi that have commonly been isolated in studies of endophytes in various substrates (e.g. cultivated fruits, leaves, roots, twigs) (Johnson *et al*., 1992; Sinclair and Cerkauskas, 1997; Taylor *et al*., 1999; Umali *et al*., 1999; Fröhlich *et al*., 2000; Shamoun and Sieber, 2000; Suryanarayanan and Vijaykrishna, 2001). Although the mechanisms of infection by fruit endophytes are still largely unknown, they are believed to colonize the fruits as early as in fruit set through vulnerable regions, such as floral remnants or pedicel tissues (Johnson *et al*., 1992). The wide occurrence of *Phomopsis* and *Colletotrichum* are consistent with the results of other studies: Shamoun and Sieber (2000) reported that *Phomopsis* was the most abundant genera of endophytes isolated from leaves and twigs of *Rubus parviflorus* and *R. spectabilis* (*Rosaceae*). Gamboa and Bayman (2001) found that *Phomopsis* and *Colletotrichum* were abundant in leaves of *Guarea guidonia* (*Meliaceae*) from 14 trees surveyed. Fröhlich *et al*. (2000) found that *Phomopsis* and *Colletotrichum* were the second and fourth most frequently recorded genera respectively from leaves of *Licuala* spp. (*Arecaceae*) in Australia and Brunei.

The proportion of fruits with no observable fungal infections reveals the effectiveness of the antifungal compounds of fruits with time. Antifungal compounds of some fruits, such as *Bridelia tomentosa*, *Diplospora dubia* and *Ilex cinerea* were effective for a very limited time, and all were soon colonized by fungi. Antifungal compounds of some fruits, such as *Ardisia crenata*, *Dichroa febrifuga*, *Sarcandra glabra*, and *Wikstroemia nutans*, were very effective for long time, so some fruits were not colonized by fungi even under incubation for 4 weeks. *Wikstroemia nutans* was an extreme case, with a very low diversity of fungi and a small proportion of fruits infected. This suggests that the antimicrobial defenses of this species are particularly effective for long period of time. The defense was developed to defend against variable surface microbes as well as to inhibit the conversion of endophytes to pathogens during ripening. This species deserves further study.

Similarities among fungi between different wild fruits

Two major clusters were generated by cluster analysis of the overall mycotas of the wild fruits (Figure 1). One cluster comprised two subclusters. The first subcluster comprised 10 fruit species, mostly with similarity values >50% and containing all of the common fungal genera on wild fruits, including *Botryodiplodia*, *Cladosporium, Colletotrichum*, *Fusarium, Penicillium, Pestalotiopsis, Phoma* and *Phomopsis.* The second subcluster comprised three fruit species that were mainly decayed by *Cladosporium*, *Fusarium* and *Penicillium.* The second cluster comprised 5 fruit species, which had very different types of fungi, but were similar in the high percentage of fruits from which no fungi were isolated. The fungal communities of two *Ardisia* species were very similar, but the other *Ardisia* species and all three *Ilex* species were no more similar than unrelated species.

The cluster analysis of the overall fungal floras of the wild fruits suggests the lack of relation with plant taxonomy. This is unexpected since related plant species have more similar defenses. The reasons for this are unknown, but it may be that the different fungal assemblage of *Ardisia quinquegona* from the other two *Ardisia* species were due to the difference in the regional pathogen pool.

Comparison of fungi on wild fruits and cultivated fruits

A comparison of fungi on cultivated fruits, and a survey of postharvest diseases on cultivated fruits in Hong Kong markets (Tang, 2000) (Table 5), show that the fungi responsible for fruit decay in nature and the postharvest diseases that develop during storage and transport are quite different. Common postharvest diseases (e.g. *Alternaria alternata, Aspergillus niger*, *Botrytis cinerea*, *Glomerella cingulata*, *Rhizopus stolonifer* and *Trichothecium roseum*), were either absent or rare on wild fruits in this study. *Botryodiplodia theobromae*, *Cladosporium* spp. and *Penicillium* spp. were the only fungi common on both cultivated and wild fruits.

Of the 55 fungal genera recorded on wild fruits, 58% are in genera that

Table 3. Taxonomic composition, diversity and richness of fungi on various fruit hosts.

	Alocasia odora	***Ardisia crenata***	***Ardisia punctata***	***Ardisia quinquegona***	***Bridelia tomentosa***	***Cleistocalyx operculatus***	***Diplospora dubia***	***Dichroa febrifuga***	***Ilex asprella***	***Ilex cinerea***	***Ilex pubescens***	***Litsea rotundifolia***	***Microcos paniculata***	***Rhodomyrtus tomentosa***	***Psychotria asiatica***	***Sarcandra glabra***	***Vibrunum sempervirens***	***Wikstroemia nutans***	**Total**
Ascomycetes	1	2	2	0	0	0	2	7	4	0	1	1	4	2	8	4	7	0	45
Coelomycetes	17	8	8	26	8	13	5	18	9	21	13	31	15	15	14	18	14	7	260
Hyphomycetes	7	7	7	0	20	12	25	0	18	8	4	2	6	10	10	3	5	1	145
Other taxa	0	2	3	2	3	5	6	1	1	1	6	0	6	3	2	4	0	0	45
Total number of fungi found	25	19	20	28	31	30	38	26	32	30	24	34	31	30	34	29	26	8	495
Number of fruits with no fungi	7	12	11	2	0	4	0	13	4	0	7	3	5	5	2	12	4	23	114
Number of fruits examined	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	540
Number of fungal species	8	10	8	4	5	13	11	11	10	19	13	16	16	10	19	10	15	3	
Shannon diversity index (H)	1.902	2.150	1.859	1.156	1.309	2.310	1.727	2.128	2.045	2.823	2.505	2.479	2.525	2.058	2.759	1.808	2.588	0.736	

Table 4. Taxonomic composition, diversity and richness of fungi on sterilized versus non-sterilized fruits.

	Alocasia odora	***Ardisia crenata***	***Ardisia punctata***	***Ardisia quinquegona***	***Bridelia tomentosa***	***Cleistocalyx operculatus***	***Diplospora dubia***	***Dichroa febrifuga***	***Ilex a sprella***

Treatment	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**
Ascomycetes	0	1	0	2	0	2	0	0	0	0	0	0	0	2	7	0	3	1
Coelomycetes	9	8	4	4	6	2	12	14	3	5	8	5	1	4	17	1	6	3
Hyphomycetes	0	7	1	6	0	7	0	0	10	10	3	9	12	13	0	0	4	14
Other taxa	0	0	2	0	2	1	2	0	3	0	4	1	6	0	0	1	1	0
Total number of fungi found	9	16	7	12	8	12	14	14	16	15	15	15	19	19	24	2	14	18
Number of fruits with no fungi	6	1	9	3	8	3	1	1	0	0	4	0	0	0	0	13	4	0
Number of fungal species	4	7	5	4	4	5	3	3	2	4	8	7	5	8	9	2	7	6
Total number of samples examined	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Shannon diversity index (H)	1.273	1.895	1.609	1.517	0.451	1.505	0.868	1.060	0.540	1.320	1.936	1.615	1.327	1.587	1.953	0.693	1.845	1.586

	Ilex cinerea		***Ilex pubescens***		***Litsea rotundifolia***		***Microcos paniculata***		***Rhodomyrtus tomentosa***		***Psychotria asiatica***		***Sarcandra glabra***		***Vibrunum sempervirens***		***Wikstroemia nutans***		**Total**
Treatment	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	
Ascomycetes	0	0	1	0	1	0	3	1	0	2	3	5	1	3	5	2	0	0	45
Coelomycetes	15	6	9	4	15	16	9	6	15	0	10	4	15	3	4	10	4	3	260
Hyphomycetes	0	8	0	4	0	2	0	6	0	10	0	10	0	3	2	3	0	1	145
Other taxa	0	1	2	4	0	0	6	0	3	0	2	0	4	0	0	0	0	0	45
Total number of fungi found	15	15	12	12	16	18	18	13	18	12	15	19	20	9	11	15	4	4	569
Number of fruits with no fungi	0	0	4	3	3	0	5	0	2	3	2	0	4	8	4	0	12	11	114
Number of fungal species	7	11	8	5	4	11	7	9	4	7	6	16	5	7	8	11	2	2	
Total number of samples examined	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	540
Shannon diversity index (H)	1.864	2.523	2.025	1.733	1.223	2.289	1.589	2.058	1.235	1.748	1.738	2.726	1.342	1.831	2.020	2.338	0.562	0.950	

s = surface sterilized, n = non-sterilized

Table 2. Occurrence of fungi on 18 fruit hosts in Hong Kong.

	Number of collections on:																																				
Fruit Species / Fungal Taxa	**AO**		**AC**		**AP**		**AQ**		**BT**		**CO**		**DD**		**DF**		**IA**		**IC**		**IP**		**LR**		**MP**		**RT**		**PA**		**SG**		**VS**		**WN**		**Total**
	s	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	
Ascomycetes																																					
Anthostomella clypeata																									1												1

Botryosphaeria sp.						1																															1
Clohiesia sp.																																	1				1
Gaeumannomyces sp.															7															1		1					9
Glomerella cingulata		1		2													3	1														1					8
Gnomonia fructicola																									1												1
Guignardia cocogena						1															1				1				3	1			2	2			11
Guignardia dioscorae																															1						1
Guignardia musae														1																							1
Lophiotrema psychotrii																												1		1							2
Massarina spp. *(4 species)*																							1			1		1					2				5
Monilinia fructicola																														1							1
Nectria spp. *(3 species)*														1																1		1					3
Coelomycetes																																					
Ascochyta sp.																										1											1
Asteroma sp.								3																1													4
Botryodiplodia theobromae	2	3			1					2							1					1			1									2			13
Cheilaria sp.																								1													1
Colletotrichum gloeosporioides	4	2			5		8	5			1					1	1			1	1		6		1		5		3		1		2	1			48
Colletotrichum musae							2	6							4		2		3																3	3	23
Colletotrichum orbiculare														1																							1
Colletotrichum spp. (2 species)																					2			2													4
Conidioxyphium sp.																								1			6		2	2				1			12
Cytosphaera sp.																			1																		1
Dinemasporium strigosum				3											2																						5
Diplodia spp. (3 species)															1				3					2										1			7
Eriospora leucostoma																															2						2
Hendersonula toruloidea																					1																1
Macrophoma spp. (3 species)							2				1													1	1		3		2								10
Menisporopsis sp.																											1										1
Monochaetia ilicina			1																																		1
Monochaetia karstenii																				1																	1
Microsphaeropsis globulosa												1																									1
Pestalotiopsis adusta																		2			1			1													4

Table 2. (continued).

	Number of collections on:																																				
Fruit Species / Fungal Taxa	**AO**		**AC**		**AP**		**AQ**		**BT**		**CO**		**DD**		**DF**		**IA**		**IC**		**IP**		**LR**		**MP**		**RT**		**PA**		**SG**		**VS**		**WN**		**Total**
	s	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	
Pestalotiopsis espaillatii																					1	1															2
Pestalotiopsis gracilis																								1													1
Pestalotiopsis kawakamii																				1																	1

Pestalotiopsis neglecta														1																							1
Pestalotiopsis phoenicis											1																		1								2
Pestalotiopsis versicolor																																		2			2
Pestalotiopsis spp. (2 species)												2														3											5
Phoma spp. (10 species)	1		1							3	3	1			1			1	2	1	2			5						1				1			23
Phomopsis archeri	2	3	1						3				1		4		2		3			2	6										1	1	1		30
Phomopsis stipata			1								1				3				2		1		3		6				2	1	6		1				27
Phomopsis spp. (2 species)															1																6	3					10
Phylctaeniella sp.																								1													1
Phyllosticta cocoicola														2					1							1											4
Pseudorobillarda sojae																				1																	1
Seimatosporium caninum				1		2					1	1																									5
Seimatosporium spp. (3 species)																				1						1								1			3
Septoria sp.															1																						1
Hyphomycetes																																					
Acremonium sp.																				1																	1
Alternaria alternata														1																						1	2
Arthrinium sp.																												1									1
Aspergillus niger																												1		1		1					3
Beltrania sp.																														1							1
Cladosporium cladosporioides						3				5			5	10			3	6		1		2		1						2		1		2			41
Dactylaria sp.																														1							1
Fusarium spp. (3 species)		3	1			3							5	2			1	3		2		1				3		1		1							26
Gliocladium solani		2											1					5		2										1			1	1			13
Gonytrichum chlamydosporium		2																																			2
Penicillium digitatum				4								7								1								4		1							17
Penicillium glabra				2																																	2
Penicillium spp. (4 species)									10	5			1																			1					17
Phaeoisaria clematidis																										1											1
Pleurophragmium sp.																										1											1
Scytalidium sp. (2 species)											3																						1				4
Stachybotrys parvispora						1														1																	2

Table 2. (continued)

	Number of collections on:																																				
Fruit Species / Fungal Taxa	**AO**		**AC**		**AP**		**AQ**		**BT**		**CO**		**DD**		**DF**		**IA**		**IC**		**IP**		**LR**		**MP**		**RT**		**PA**		**SG**		**VS**		**WN**		**Total**
	s	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	**s**	**n**	
Ulocladium sp.																										1											1
Zygosporium gibbum												2										1		1				3		2							9
Myxomycetes																																					
Physarum nutans																1																					1

Stemonitis sp.																				1																	1
Oomycetes																																					
Phytophthora-like sp.											1	1																									2
Others																																					
Sterile mycelium spp. (10 species)			1		2	1	1		1		2		6				1					4			4				2								25
Unidentified spp. (10 species)			1				1		2		1										2				2		3				4						16
Total number of fungi	9	16	7	12	8	12	14	14	16	15	15	15	19	19	24	2	14	18	15	15	12	12	16	18	18	13	18	12	15	19	20	9	11	15	4	4	495
Number of fruits with no fungi	6	1	9	3	8	3	1	1	0	0	4	0	0	0	0	13	4	0	0	0	4	3	3	0	5	0	2	3	2	0	4	8	4	0	12	11	114
Total number of samples examined	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	540

AO = *Alocasia odora,* AC = *Ardisia crenata,* AP = *Ardisia punctata,* AQ = *Ardisia qinquegona,* BT= *Bridelia tomentosa,* CO = *Cleistocalyx operculatus* DD = *Diplospora dubia*, DF = *Dichroa febrifuga*, IA = *Ilex asprella*, IC = *Ilex cinerea*, IP = *Ilex pubescens*, LR = *Litsea rotundifolia*, MP = *Microcos paniculata*, RT = *Rhodomyrtus tomentosa*, PA = *Psychotria asiatica*, SG = *Sarcandra glabra*, VS = *Vibrunum sempervirens*, WN = *Wikstroemia nutans*

s = surface sterilised fruits, n = non-sterilised fruits

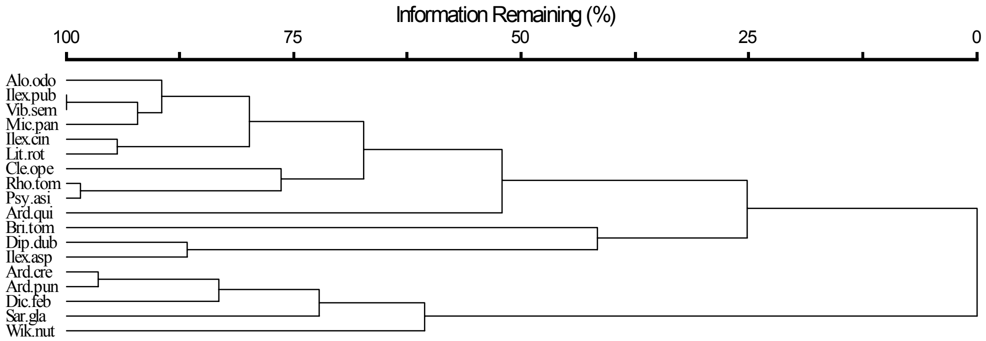

Fig. 1. Cluster analysis of the overall fungal floras of the wild fruits.
(Alo.odo = *Alocasia odora*, Ard.cre = *Ardisia crenata*, Ard.pun = *Aridsia punctata*, Ard.qui = *Ardisia quinquegona*, Bri.tom = *Bridelia tomentosa*, Cle.ope = *Cleistocalyx operculatus*, Dip.dub = *Diplospora dubia*, Dic.feb = *Dichroa febrifuga*, Ilex.asp = *Ilex asprella,* Ilex.cin = *Ilex cinerea*, Ilex.pub = *Ilex pubescens*, Lit.rot = *Litsea rotundifolia*, Mic.pan = *Microcos paniculata*, Rho.tom = *Rhodomyrtus tomentosa*, Psy.asi = *Psychotria asiatica*, Sar.gla = *Sarcandra glabra*, Vib.sem = *Viburnum sempervirens*, Wik.nut = *Wikstroemia nutans*.)

have not been recorded on cultivated fruits or previous surveys of fungi onfruits (Wun, 1991; Wright, 1998; Tang, 2000). Examples included the commonly occurring ascomycetes *Massarina* and *Gaeumannomyces*, and the anamorphic fungi *Conidioxyphium*, *Diplodia*, *Macrophoma*, *Seimatosporium*, *Stachybotrys* and *Zygosporium*.

Wun (1991) carried out a field survey on the surface microbes and endophytes of *Citrus* spp. in Tai Po Kau Citrus Orchard (Table 6). She found that *Cacumisporium* spp., *Brachysporium* and *Cladosporium* spp. were the most frequently recorded surface microbes and *Colletotrichum* spp., *Nigrospora* spp. and *Trichophyton* spp. were the most frequently recorded endophytes. These species were not found in this study except *Cladosporium* spp. and *Colletotrichum* spp. This indicates that fungi responsible for decay on wild fruits are quite different from cultivated fruits.

The divergence in fungal communities between wild and cultivated fruits presumably reflects their different characteristics. Wild fruits are relatively higher in fiber, lower in soluble carbohydrates, and are more resistant to diseases than cultivated fruits. Cultivated fruits have resulted from human-directed selection for taste and are grown in controlled conditions with application of pesticides and fungicides, while the wild fruits are selected by natural processes and grow under natural seasonal cycles (Billings, 1997). The practice of monoculture makes cultivated fruits more susceptible to infection by virulent pathogens than wild fruits, while diseases of wild fruits are rarely widespread, because a balance between host and pathogen has naturally evolved in the wild (Shivas and Hyde, 1997). Most decay of wild fruits can be attributed to the non-latent opportunistic fungi that attack overripe or susceptible fruits.

Table 5. Postharvest diseases recorded from commercial fruits and vegetables of Hong Kong (Tang, 2000).

Anamorphic fungi	
Alternaria	*Fusarium*
Aspergillus	*Geotrichum*
Cercospora	*Penicillium*
Chlamydomyces	*Pestalotiopsis*
Cladosporium	*Phomopsis*
Colletotrichum	*Trichothecium*
Curvularia	*Verticillium*
Dreschlera	
Zygomycete	
Rhizopus	

Table 6. Species recorded from citrus fruits of Hong Kong (Wun, 1991).

Ascomycete	**Zygomycete**
Cunninghamella	*Mortierella*
Guignardia	*Mucor*
Saccharomyces	*Rhizopus*
Anamorphic fungi	
Acremonium	*Humicola*
Alternaria	*Idriella*
Aspergillus	*Memnoniella*
Botrytis	*Microsporum*
Brachysporium	*Monilia*
Cacumisporium	*Nigrospora*
Chalara	*Paecilomyces*
Colletotrichum	*Penicillium*
Curvularia	*Sphaceloma*
Dreschlera	*Trichoderma*
Epicoccum	*Trichophyton*
Fusarium	

Possible types of fruit fungi

Observations were made in the field and the laboratory in order to establish the possible types of fungi that significantly affect the relationships between wild fruits and frugivores. Microbial attack on mature fruits presumably affects not only the frugivores that consume fruits as part of their diet, but also fruiting plants that rely on frugivores for seed dispersal. Thus, "ecologically significant fungi" are those that reduce the chances of fruits being consumed and seed dispersed. During the survey, three types of fruit fungi were identified. These included: pathogens, latent opportunists and non-latent opportunists.

Pathogens

Although fungal pathogens are very diverse on cultivated fruits, they are comparatively less common and rarely damaging on wild fruits in the natural ecosystems, where a balance between hosts and pathogens has evolved (Shivas and Hyde, 1997). Wild fruits, in general, have apparently developed abilities to defend against pathogens to retain attractiveness for dispersal. Selective pressures are believed to have been exerted on plants favouring the evolution of plant traits enhancing seed dispersal (Herrera, 2002). Pathogenic infections on unripe wild fruits are therefore relatively rare in nature and most diseases on ripe wild fruits are caused by non-latent opportunists.

Latent opportunists

Latent opportunists (endophytes) are fungi that colonize and cause unapparent, asymptomatic infections in healthy plant tissues (Wilson, 1995). Latent infections are initiated at any time from petal fall to ripening, but the germinated spores only produce appressoria which remain latent for a few days or weeks to several months until the climacteric onset of ripening (Coates *et al*., 1993). Two important changes "re-activate" the fungal appressoria within cultivated fruits: one is the disappearance of fungistatic compounds present in the fruit skin and pulp in the advance of maturity; the other is the availability of more soluble carbohydrates during ripening (Parbery, 1996). Several genera of fungi, notably *Colletotrichum*, *Diplodia*, *Guignardia*, *Pestalotiopsis*, *Phoma* and *Phomopsis,* are responsible for latent infections of fruits. It has been suggested that all cultivated fruits in the tropics, including citrus, mangoes, papayas, avocados, bananas, have latent infections of *Colletotrichum gloeosporioides* in the peel at harvest (Meredith, 1964; Wun, 1991; Coates *et al*., 1993; Wright, 1998).

In the current survey, it was found that *Colletotrichum* and *Phomopsis* were the most abundant endophytes. They were found in 15 out of 18 fruit species studied. Although they occurred widely in wild fruits, decay caused by latent fungi is relatively less important in the wild than in cultivation, where cultivars have been selected for high palatability and thus low defenses (Cipollini and Stiles, 1993b). Latent opportunists, in general, are unlikely to develop until after the fruits would normally have been dispersed. They can only cause diseases within fruits when the fungistatic compounds disappear from the skin and pulp of un-dispersed overripe fruits. Un-dispersed fruits are more noticeable in under-storey shrubs than open-slope shrubs. For example, it was noticed that a lot of fruits of *Alpinia chinensis* on Mount Nicholson remain un-dispersed and rot.

Fast-colonizing opportunists

This type of fungi is characterized by fast establishment on wounds or susceptible regions of fruits. Several genera, notably *Aspergillus*, *Botrytis*, *Cladosporium, Fusarium*, *Geotrichum*, *Penicillium*, *Rhizopus*, have been recognized as wound-invading parasites (Eckert *et al*., 1975). Some of them are capable of producing mycotoxins during growth, such as patulin in *Cladosporium* and *Penicillium*, nivalenol in *Fusarium* and aflatoxins in *Aspergillus*. They have been proved to be highly deterrent to birds and certainly exacerbate negative effects on seed dispersal (Borowicz, 1988; Buchholz and Levey, 1990; Cipollini and Stiles, 1993a). They are very likely to be important on ripe fruits before their dispersal.

In this survey, insect puncture of fruits was very common in the field. The presence of insect punctures may encourage the growth of non-latent opportunists on some species of fruits, depending on the ability to heal wounds and the effectiveness of the fungistatic compounds at different ripening stages. It was observed that *Alocasia odora*, *Ardisia crenata*, *A. punctata*, *Bridelia tomentosa*, *Diplospora dubia*, *Ilex cinerea*, *Psychotria asiatica* had many insect punctures on the skin. Some of the wounds were healed but some did not. Clear insect punctures and the associated fungal colonization by non-latent opportunists were observed on *Diplospora dubia*. Other invasions may be on intact skin due to decline in the chemical defense system.

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