LIFE TABLE STUDY OF *PENTALONIA NIGRONERVOSA* & *P. CALADII* ON BANANA & VECTOR TRANSMISSION OF *BANANA BUNCHY TOP VIRUS* (BBTV)

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DEDICATION

Nandy, thank you for teaching me how to fish.

Love you.

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Abstract

Pentalonia nigronervosa, the banana aphid, (Hemiptera: Aphididae) vector-transmits *Banana bunchy top virus* (BBTV): the greatest constraint for banana (*Musa* sp.) crop production in Hawaii. Prior to 2010, *Pentalonia caladii*, the cardamom aphid, was regarded in synonymy with *P. nigronervosa*, therefore, earlier studies of *Pentalonia* aphids may not accurately reflect the true nature of each species. This research compares the life history, colonization ability and transmission competence of genotypically distinct *P. nignonervosa* and *P. caladii* asexual lineages collected from taro, heliconia, red ginger and banana using banana leaf discs or plants. Life history and developmental parameters were similar for both species when reared on banana leaf discs, but *P. caladii* from taro colonized banana plants with difficulty. All lineages of *P. caladii* vectored BBTV in leaf disc transmission assays, but only the *P. caladii* lineage from taro and *P. nigronervosa* from banana were able to vector BBTV to plantlets.

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Chapter 1: Introduction

Aphids are small, soft-bodied phytophagous insects belonging to the superfamily Aphidoidea. The family Aphididae includes 4400 species grouped within 8 sub-families. Aphids are classified within the order Hemiptera suborder Sternorrhyncha, along with scales, psyllids and whiteflies (Blackman and Eastop, 2000).Aphids have a simple body plan, yet, exhibit complex feeding and reproductive strategies. Some aphid species are capable of exploiting several plant species, withstanding a wide variety of climates and temperatures, and transmitting plant viruses that limit crop production. A few aphid species are known to contain host plant races, or biotypes that differ in morphology or fitness across host plants. Research that focuses on the ecological and biological specialization of aphids as pests may help to establish more sustainable management practices.

Hawaii leads the nation in fresh market banana production and banana (*Musa* sp.) is the state's 3rd largest fruit crop (NASS, 2010). In 2008, banana growers produced 17.4 million pounds of bananas on 1,300 acres with an estimated market value of $8 million (NASS, 2009). If compared to 2007, crop production and market values were reduced by 32% and 24%, respectively (NASS, 2009). It is believed a disease caused by *Banana bunchy top virus* (Nanoviridae: Babuvirus), referred to as banana bunchy top disease (BBTD), has contributed to the recent reduction in the number of farms and acreage being devoted to banana production although, unfortunately, there is still a lack of statistical data to support these claims.

BBTD is the most destructive disease affecting banana plants in Hawaii and in several sub-tropical and tropical regions of the world (Dale et al., 1992). Banana bunchy top virus (BBTV) reduces plant growth and fruit production resulting in unmarketable harvests, and ultimately kills banana plants. BBTV is spread by the banana aphid, *Pentalonia nigronervosa* (Magee, 1927). Recently, Foottit et al (2010) separated the "banana aphid" into *P. nigronervosa* and *P. caladii*, based on morphological and molecular characters. *Pentalonia caladii* is also suspected to vector BBTV (Foottit et al., 2010). Detailed information on the ability of *P. caladii* to colonize, reproduce on banana and transmit BBTV will advance our awareness, and lead to more effective management practices for banana production.

Aphid feeding

Aphids prefer to feed on the phloem and less commonly on the xylem of herbaceous shrubs, trees, weeds and cultivated plants (Blackman and Eastop, 2000, Klingauf, 1987). According to Blackman and Eastop (2000), aphids feed on nearly every cash crop. Aphids can feed on almost all parts of plants, including the roots and the bark of trees (Guerrieri and Digilio, 2008). Aphid feeding can affect plants directly and indirectly. Aphid feeding can lead to stunting, malformation, galling, chlorosis and necrosis of plant tissues (Goggin, 2007, Guerrieri and Digilio, 2008). Salivation also enables the inoculation of plant pathogenic viruses (Gray and Gildow, 2003). Additionally, aphid excretion (honeydew) can indirectly inhibit photosynthesis by facilitating fungal growth on the surface of the plant tissues (Goggin, 2007).

Aphids have piercing sucking mouthparts specialized to extract sap from plant tissues. Aphid mouthparts comprise a maxillary stylet and a mandibular stylet both held within a modified labium and labrum (Klingauf, 1987). The maxillary stylet contains a salivary and a food canal. When an aphid probes on a potential host plant it detects secondary plant metabolites that enable host plant recognition (Dixon, 1998). Chemoreceptors located within the epipharygeal organ (Anderson and Bromley, 1987) are responsible for host plant recognition and acceptance (Powell et al., 2006).

Many aphids have co-evolved with their host plants (Dixon, 1998, Powell et al., 2006) and therefore may have specific stylet adaptations (Guerrieri and Digilio, 2008). The ability of aphids to feed on certain host plants can often be predicted by examining the distance of the sieve tubes from the surface of the plant and the length and width of the stylet (Guerrieri and Digilio, 2008, Will and van Bel, 2006). The majority of aphid species feed on one (monophagy) or on a limited number of plant species (oligophagy), while the few generalists colonize a wide range of hosts (polyphagy).

Aphid reproduction

The majority of aphid species reproduce through cyclical parthenogenesis, i.e. the alternation of sexual and several asexual reproductive cycles. Cyclical parthenogenesis evolved in the temperate regions where many aphid species are known to have radiated (Blackman and Eastop, 2000). During asexual reproduction, aphids combine both viviparity (live birth) and parthenogenesis (reproduction without the fusion of gametes) (Blackman and Eastop, 2000). Live birth is believed to be a derived character, although aphids started producing unfertilized eggs through parthenogenesis about 200 million years ago (Dixon, 1998). Live birth through parthenogenesis has allowed the development of "telescoping of generations" where each aphid is born with developing embryos within it (Blackman and Eastop, 2000, Dixon, 1998). In the spring and summer asexual reproduction dominates, but in the fall aphids from temperate regions can produce fertilized eggs that persist through harsh winters (holocycly). In some holocyclic species, changing environmental conditions trigger host plant alternations (heteroecy). Summer prompts aphids to leave the primary (woody) host to a secondary (herbaceous) host where reproduction continues through parthenogenesis. Autumn signals aphid sexual morph production and the return to the primary host plant where fertilized eggs overwinter (Williams and Dixon, 2007).

The differentiation of sexual forms in most aphid species requires seasonal changes (decrease in day length and/or gradual drop in temperature); therefore, it is extremely rare for sexual forms to occur in tropical regions (Dixon, 1998). Within the tropical belt, where environmental conditions are fairly constant, aphids generally do not lay eggs (anholocycly), but reproduce asexually through apomictic parthenogenesis. Aphids in Hawaii are believed to reproduce exclusively through obligate parthenogenesis. However, the presence of males of *Greenidea formosana* has been recorded from Hawaii Volcano National Park, Hawaii island (Gruner, 2004).

Asexual lineages

Exclusive asexual reproduction prevents gene recombination; however asexually reproducing aphids can mutate, therefore the term “asexual lineage” should be used to denote asexually reproducing lineages, instead of “clonal lineage” (Loxdale, 2008). Mutations can create genetic variation within aphid populations on a local scale (Loxdale, 2008). Genetic differences may have an effect on behavior, including host finding and feeding preferences (Symmes and Perring, 2007, Margaritopoulos et al., 2007). For instance, asexual lineages of the melon aphid, *Aphis gossypii*, the greenbug *Schizaphis graminum*, and the bird cherry oat aphid, *Rhopalosiphum padi* have been shown to vary in host preference (Bencharki et al., 2000, Sadeghi et al., 1997, Najar-Rodríguez et al., 2009, Charaabi et al., 2008, Gray et al., 2002). The green peach aphid, *Myzus persicae* contains host plant associated forms that can be distinguished by the length of the ultimate rostral segment (Margaritopoulos et al., 2007). Specific color morphs have also been documented according to host preference or seasonal temperature changes (Dedryver et al., 2005, Braendle and Weisser, 2001). Asexual lineages of *M. persicae nicotianae*, which are adapted to colonize tobacco, vary in color and content of esterase enzymes from biotypes of *M. persicae* colonizing alternative host plants (Margaritopoulos et al., 2007).

Many differences between asexual lineages appear to be associated with plant use. Genotypically distinct *A. gossypii* display host preference and a higher fitness on the host of origin (Najar-Rodríguez et al., 2009). Beregovoy and Peters (1996) found *S. graminum* biotypes capable of exhibiting K- or r-strategies depending on the host plant they developed on. Alates of two distinct *A. gossypii* lineages preferred original host plant odors when given a choice between the original and an alternative host plant (Najar-Rodríguez et al., 2009) *Aphis gossypii* host races from melon and cotton failed to reproduce when reared on alternative host plants (Najar-Rodríguez et al., 2009).

Virus transmission

Aphids cause major economic injuries to commercial crop production and reduce market values mostly through transmission of plant viruses. Aphids vector over 50% of the 550 known insect-transmitted plant viruses (Nault, 1997, Brunt, 1996 , Link and Fuchs, 2005). *Aphis gossypii* and *M. persicae* transmit more than 100 and 50 different phytopathogenic viruses, respectively (Blackman and Eastop, 2007).

Because aphids are known to evolve host races, it is also probable that certain aphid genotypes may be better adapted to transmit plant viruses than others (Symmes and Perring, 2007). Transmission efficiency between aphid asexual lineages may be relative to differences in fitness, virus type, or host plant origin (Dedryver et al., 2005). Virus specificity seems to be regulated at the molecular level. In fact, viral particles must recognize and bind at specific receptors in the vector; otherwise the virus would pass through the digestive system and be excreted with honeydew (Hull, 2002, Link and Fuchs, 2005).

Virus transmission by arthropods is categorized according to the amount of time the virus is retained after acquisition and the location of viral attachment within the vector (Fereres and Moreno, 2009). Currently, the transmission of plant viruses by arthropod vectors is divided into 4 categories: non-persistent (stylet-borne), semi-persistent (foregut-borne), persistent circulative and persistent propagative (Fereres and Moreno, 2009). Aphids transmit non-persistent viruses during probing tests. When an aphid lands on a plant and detects a smooth surface it attempts to probe on the surface of the plant tissues, usually for less than a minute (Powell et al., 2006). Within this short period, stylet-borne viruses may be injected into the plant cells through salivation. Persistent viruses generally cross the epithelial tissues of the gut and salivary glands, and are released through salivary secretions (Fereres and Moreno, 2009). Circulative and propagative viruses take a much longer time to be acquired and inoculated, because they are generally associated with the phloem tissues; however, the virus will persist throughout the vector’s lifetime. Propagative viruses may even be maintained throughout other vector generations’ transovarially (Fereres and Moreno, 2009). Examples of aphid-transmitted non-persistent, semi-persistent, and persistent viruses spread in Hawaii are *Papaya ringspot virus* (PRSV), *Cucumber mosaic virus* (CMV) and *Banana bunchy top virus* (BBTV), respectively.

Banana

The family Musaceae includes two genera; *Musa* (banana) and *Ensete* (false banana) (Jones, 2000). The family Musaceae is closely related to other families of the order Zingiberales: Strelitziaceae, Heliconiaceae, Zingiberaceae, Cannaceaea, Costaceae, Lowiaceae, and Marantaceae. The banana plant is a perennial herb made up of a pseudostem from which new leaves emerge (leaf sheaths), an underground corm or "mat" from which "keiki" or new shoots form, a stem, a flower and bunch from which bananas can be harvested when a plant reaches maturity (Gold et al., 2002, Jones, 2000)

Banana is grown in more than 120 countries and is an important staple crop to millions of people (Jones, 2000). Written records of banana cultivation date to 500-600 BC in India, although crop production of bananas is believed to have occurred longer than 10,000 years ago (Dale, 1987). In Hawaii, approximately 100 banana varieties have been grown and the use of many cultivars date back to the Pre-Polynesian settlers (Kepler and Rust, 2011, Pope, 1926) who grew bananas in traditional Hawaiian farms for nearly 1800 years (Kepler and Rust, 2011). The banana plant has been used to make clothing, shoes, line houses and ovens, in medicinal remedies, as religious offerings, and even in common colloquial expressions (Nelson et al., 2006, Wagner et al., 1999).

Banana bunchy top virus (BBTV)

BBTV was first recorded from Fiji in 1889, although the virus is believed to have originated in South Asia, where many plants of the genus *Musa* originated (Magee, 1940). In 1913, BBTV was reported in Egypt (Magee, 1927), then Sri Lanka and Australia (Magee, 1953). In the 1920's the use of BBTV-infected planting material resulted in production losses up to 95% in some banana growing areas of Australia (Magee, 1927). BBTV has now spread throughout the Pacific-Asia region (Ploetz, 2003) including China, Indonesia, the Philippines, Vietnam, India, Pakistan, Taiwan, Hawaii, Tonga, Western Samoa, and Guam. The virus is also spread in some countries in Africa (Ploetz et al., 2003). Central and South America, the Caribbean, Papua New Guinea, and Thailand are devoid of the virus (Dale et al., 1992).

In Hawaii, BBTV was first reported in 1989 on the Island of Oahu (Conant, 1992) and has since spread to Hawaii (1995), Kauai (1997), Maui (2002), and Molokai (2005) (Nelson, 2006, Kepler and Rust, 2011) most likely through the movement of infected plant material (Almeida et al., 2009). In Hawaii, BBTV alerts have been issued to the public through local newspapers, bulletins, educational videos, and even a website where the public can access information on several aspects of BBTV management (Nelson and Richardson, 2006, Nelson, 2006). After quarantine efforts failed to contain the virus, rouging of infected plants, vector management and proper sanitation became the most common practices for BBTV management.

BBTV is known to affect all banana varieties; however, differences in susceptibility have been recorded, especially within the ABB subgroup (Hooks et al., 2009b, Wardlaw, 1972, Dale et al., 1992). Banana cultivars known to be susceptible to BBTV are the edible bananas belonging to: *Eumusa, Australimusa, M. balbisiana, M. velutina, M. coccinea, M. jackeyi, M. ornata, M. acuminate* spp. *Zebrine,* and *Ensete ventricosum* (Ploetz et al., 2003). Other known viruses affecting banana include *Cucumber mosaic virus* (CMV) which occurs in most production regions, *Banana streak virus* (BSV) spread in Africa, and *Banana bract mosaic virus* (BBrMV) in the Philippines. However, the most economically damaging virus is BBTV (Dale et al., 1992).

BBTV is an ssDNA virus made up of 6-9 circular components (Fauquet et al., 2005) comprised of approximately 1000 nucleotides each. The genomic DNA is contained within non-enveloped icosahedral virions of 18-20 nm in diameter (Wu and Su, 1990a, Xie and Hu, 1995). BBTV can move systemically infecting the entire banana mat and offshoots (keiki or suckers). BBTV infection causes fruit distortion and dwarfing (Dale and Harding, 1998). Typical bunchy top symptoms of banana plants include: chlorosis of leaf margins, narrowing and bunching of successive leaves, “morse code” dashes, hooking along the midrib of the leaves, and dark green streaking of the petioles (Thomas et al., 1994, Dale et al., 1992). In the field, symptoms are expressed 25-85 days after initial infection (Hooks et al., 2008). *Pentalonia nigronervosa* is able to acquire and subsequently transmit BBTV to new plants, even if it feeds on a plant treated with a bananacide - a registered herbicide for killing banana plants (Hooks et al., 2009a). Magee

(1940) found that when BBTV infected leaves are excised from the plant, they retained infectivity for at least 12 days.

No alternative host plants have been recorded for BBTV. *Colocasia sp.* was reported to harbor BBTV non-symptomatically (Ram and Summanwar, 1984), although this has been refuted (Hu et al., 1996). Canna, cucumber, tobacco, chenopodium, and cotton have all been tested for BBTV infection with negative results (Yasmin et al., 2001). Researchers in Australia failed to vector transmit BBTV to *Alocasia brisbanensis, Alpinia arundelliana, Alpinia caerulea, Alpinia zerumbet, Canna indica, Colocasia esculenta, Hedychium coronarium* and *Heliconia psittacorum* cv. Red Parakeet (Geering and Thomas, 1997). Also, no BBTV symptomatic plants were found during extensive visual surveys across plants of the families Araceae and Zingiberaceae including *Alocasia brisbanensis, Alpinia caerulea, Canna indica, Canna* x *generalis, Canna* x *orchiodes, Colocasia esculenta* and *Strelitzia reginae* that were sampled in proximity to BBTV infected banana fields (Geering and Thomas, 1997).

BBTV is spread through aphid transmission and through planting of infected suckers (Dale et al., 1992, Magee, 1927). A few aphid species that colonize banana plants, including *M. persicae, A. gossypii,* and *R. padi* have been tested for BBTV transmission, with negative results (Dale, 1987, Patel and shukla, 2009, Hu et al., 1996, Yasmin et al., 2001). Magee (1927) was the first researcher who demonstrated that *P. nigronervosa* is the vector of BBTV. *Pentalonia nigronervosa* transmits BBTV persistently. Adults are more efficient than 3rd instar nymphs (Anhalt and Almeida, 2008). BBTV persists through vector molting (transdatial passage) with no transovarial transmission reported (Magee, 1927). BBTV may be acquired in a minimum acquisition access period of 4 hours and a minimum inoculation access period of 15 minutes (Hu et al., 1993). The minimum latent period for transmission in the aphid is 20-28 hours (Anhalt and Almeida, 2008). If infective aphids are removed from BBTV infected material they remain infective for at least 84 hours (Magee, 1927). BBTV has been localized within the anterior midgut and principal salivary glands of *P. nigronervosa* (Bressan and Watanabe, 2011). Understanding how viruses are acquired, how they travel within a vector, and how they are inoculated may enable the development of novel strategies to block or manipulate viral movement thus preventing virus transmission.

The genus Pentalonia

The genus *Pentalonia* includes: *Pentalonia gavarri* found on grasses in the Philippines and Malaysia, *Pentalonia kalimpongensis* found on Zingiberaceae plants in India, and *Pentalonia nigronervosa,* distinguished in 2 forms: “typica” and “caladii”, found throughout tropical and subtropical regions (Blackman and Eastop, 2006, Foottit et al., 2011). *Pentalonia nigronervosa* is known to reproduce by obligate parthenogenesis throughout its host range, with the exception of a report from West Bengal (India) where an oviparous female with offspring was collected from turmeric (Zingiberales, *Curcuma domestica*) (Blackman and Eastop, 2006, Bhanotar and Ghosh, 1969).

Pentalonia nigronervosa seeks refuge among leaf sheaths and at the base of the pseudostem (Wardlaw, 1972, Magee, 1927). The aphid is usually ant attended(personal field observations of banana, red ginger, heliconia, and taro) however, ant presence on banana is not a prerequisite for aphid presence (Hooks et al., 2011). *Pentalonia nigronervosa* displays host plant preferences and may colonize non preferred host plant species with difficulty (Ploetz, 2003). In the past, *P. nigronervosa* form “typica” and form “caladii” have collectively been referred to as the banana aphid. In most tropical regions the “banana” aphid has been collected from plants in the family Araceae (*Colocasia* sp., *Xanthosoma* spp., *Dieffenbachia* spp., *Caladium* spp.), Musaceae (*Musa* spp.), Zingiberaceae (*Zingiber* spp., *Alpinia* spp., *Hedychium* spp., *Costus* spp., *Elettaria* spp.), Heliconiaceae (*Heliconia* spp.) and Cannaceaea (*Canna* spp.) (Waterhouse, 1987, Blackman and Eastop, 2000). Some authors have separated the taxa based on form “nigronervosa” and form “caladii” (Viswanathan and Regupathy, 1992, Rajan, 1981, Venugopal et al., 1999).

Padmalatha Singh (2001, 2002b) differentiated antennal characteristics between alates from *Musa* sp. and *Colocasia* sp. They found the secondary sensoria, the number of sensory hairs on the antennae and total length of the antennae showed variation between aphids collected from different hosts (Padmalatha and Singh, 2002b). Further, it was found that aphids collected from *Costus* sp., *Anthurium* sp., *Musa* sp., and *Colocasia* sp. differed in color and that ultimate rostral length and total body length are longer for aphids collected from *Musa* sp. (Padmalatha and Singh, 2001). Aphids collected from *Colocasia* sp. and from *Musa* sp. were also differentiated by the total number of hairs present on rostral segments, 2-3 to 3-4, respectively (Padmalatha and Singh, 2002b).

Recently, Foottit et al (2010) used morphological and molecular characters to differentiate *Pentalonia* aphids colonizing plants in the family Musaceae and Araceae/Zingiberaceae and found the ultimate rostral segment of aphids from *Musa* sp. do not overlap with aphids from alternate hosts (Foottit et al., 2010). In addition, mitochondrial COI gene sequences separated *Pentalonia* aphids collected from *Musa* sp. from aphids of alternative host plants of the family Zingiberaceae and Araceae (Foottit et al., 2010). These morphological and genetic differences led the researchers to propose 2 species; *P. nigronervosa* Coquerel and *P. caladii* van der Goot (Foottit et al., 2010).

Pentalonia caladii, the cardamom aphid, is viviparous, and colonizes *Cardamom* sp., *Colocasia* sp., *Alocasia* sp., and *Caladium* sp. (Rajan, 1981, Siddappaji and Reddy, 1972) and many other plant genera within the family Zingiberaceae and Heliconiaceae (Galambao, 2011), although the host range is probably wider than previously thought. *Pentalonia caladii* transmits *Cardamom bushy dwarf virus* (Nanoviridae: Babuvirus), the etiological agent of Foorkey Disease of cardamom (Mandal et al., 2004, Tidona and Darai, 2011), in a persistent manner. *Pentalonia caladii* also transmits cardamom vein clearing to large Cardamom (Rajan, 1981, Venugopal, 1995). Both species of *Pentalonia* along with 9 other aphid species are able to transmit “Katte” mosaic virus to cardamom (Rao and Naidu, 1974, Varma and Capoor, 1958). *Pentalonia nigronervosa* is also known to vector transmit *Abaca bunchy top virus* to *Musa textilis (Tidona and Darai, 2011),* however there is no data available on vector transmission by *P. caladii*. In fact, most studies on *P. caladii* biology and ecology is relatively limited outside of India.

Most studies on the biology and fitness characters of *Pentalonia* aphids have focused on one asexual lineage or form (Anhalt and Almeida, 2008, Rajan, 1981, Viswanathan and Regupathy, 1992, Robson et al., 2007). Recently, Bhadra and Agarwala used a colony of *Pentalonia* aphids derived from *Musa* sp. and a colony from *Colocasia* sp. and compared their fitness characters on their original host plants (2010). When transferred to *Colocasia* sp., aphids from *Musa* sp. formed colonies but population levels remained low. These findings were similar to those of Lomerio and Calilung (1993), who evaluated the developmental parameters of banana aphids, collected from banana and abaca, on five hosts. Incorporating data from whole plants and leaf disc assays the same authors found that aphids from *Colocasia* sp., when transferred to *Musa* sp., remained only at low levels and eventually died out.

In Hawaii, information about the population genetics of *P. nigronervosa* and *P. caladii* is now available (Galambao, 2011). Through the use of microsatellite markers, the diversity in *Pentalonia* aphid populations was described for the first time. *Pentalonia nigronervosa* and *P. caladii* contained 52 and 38 genotypes, respectively (Galambao, 2011). Island and host plant significantly affected the distribution of those genotypes. 87% of colonies of *P. nigronervosa* preferentially colonized plants of the family Musaceae, whereas 79% of colonies of *P. caladii* preferentially colonized plants of the family Zingiberaceae (Galambao, 2011). In Hawaii, the bacterial community associated with *P. caladii* has been determined from aphids collected from taro and red ginger (Jones et al., 2011). *Pentalonia caladii* from both host plants were found to contain the secondary symbiont *Wolbachia* (Jones et al., 2011). It is currently unknown if *Wolbachia* is also found within *P. nigronervosa*. The existence of *Wolbachia* within aphids is rare and the presence of this symbiont is not quite understood. Recent findings suggest secondary symbionts influence aphid fitness on particular host plants, provide protection against parasitoids and fungus (Ferrari et al., 2004), and lessen effects of high temperature on fitness (Russell and Moran, 2006) It is possible that *Wolbachia* may likewise influence aphid biology. At this time, it is unknown if secondary bacteria affect virus transmission, although the aphid primary symbiont *Buchnera aphidacola* produces symbionin, a protein of the family chaperonins, which is known to stabilize circulatively transmitted plant viruses in the aphid haemocoel (Hogenhout et al., 2008).

BBTV management

The biology, ecology and vector competency of a pest help us to further understand how we can develop more informed management decisions. The majority of research focusing on plant-virus-vector interactions, epidemiology and control of BBTV has been conducted in Australia, India, the Philippines and Hawaii. The management of BBTV relies on proper sanitation, rouging and removal of infected material, and aphid management. Upon detection of BBTV in a region, efforts are often launched to eradicate the disease, with varying levels of success. The incidence of BBTV in Hawaii has reached such proportions that it is no longer feasible to conduct eradication programs. Once a plant is infected, even if it is treated with a bananacide the virus within the plant retains its infectivity for up to 6 weeks, enabling further BBTV spread through the insect vector (Hooks et al., 2009a). Therefore, it is essential to remove rouged dead plants.

Aphid populations can also be suppressed by the action of parasitoids and predators. In Hawaii, Braconid and Aphelinid parasitoid wasps are known to attack *Pentalonia* aphids in the field (Muratori et al., 2009). However, because *Pentalonia* aphids are cryptic in nature, these parasitoids may not be entirely effective. Recently, Muratori et al.(2009) discovered the fly *Endaphis fugitiva* (Cecidomyiidae) parasitzes *Pentalonia* aphids. *Endaphis fugitiva* fly larvae that are deposited on the plant surface actively search for their aphid host. These larvae are small, mobile and are potentially able to access areas where aphids prefer to feed that may be inaccessible to other larger flying parasitoids (Muratori et al., 2009). The use of *E. fugitiva* as a biological control agent for *Pentalonia* aphids seems plausible because this species is already found in Hawaii and is known to also target *A. gossypii* (Muratori et al., 2009).

Other long term management options include manipulating plants to make them resistant to BBTV. Transgenic plants that are resistant to BBTV have been developed by researchers at the University of Hawaii at Manoa (Borth et al., 2011). Developing, permitting and implementing transgenic plants in the field is a lengthy process, therefore transgenic BBTV resistant banana plants may not be available for another 10-20 years (Wayne Borth, personal communication). The augmentation of biological control agents and the development of transgenic banana show promise to reduce *Pentalonia* populations and decrease the spread of BBTV.

Structure of thesis

Control of aphid vector populations is a major component in managing BBTV. Fundamental knowledge about aphids that vector BBTV and transmission dynamics will improve our ability to manage the disease and increase banana production. The recent elevation to species status of *P. nigronervosa* form “caladii” has prompted the need for more detailed information on the ability of both species to colonize banana and transmit BBTV. I have performed experiments using 12 asexual lineages collected from 4 different host plants: banana, red ginger, heliconia and taro. Testing multiple asexual lineages of both *Pentalonia* species may unveil intra or inter-specific differences and lead to more accurate predictions of aphid fitness and vector competence.

In chapter 2, I present data on the biology of multiple asexual lineages of *P. nigronervosa* and *P. caladii*. Representative life tables for 12 asexual lineages were constructed and population parameters were calculated. Developmental data were also recorded. In addition, I also determined the feeding sites of *P. caladii* on entire potted banana plants. The goal of this study was to evaluate if *P. caladii* asexual lineages were capable of living and reproducing on banana.

The banana aphid causes minimal damage through feeding; therefore, understanding the biology of these aphids is primarily important if their ability to vector BBTV is considered. In Chapter 3, I focus on the vector transmission of BBTV by the 12 asexual lineages of *Pentalonia* previously evaluated for fitness parameters. There is no current information on the ability of *P. caladii* to transmit BBTV. Understanding the vector competence of this species is important for banana growers and may lead to changes in sanitation practices, which may include the removal of alternative hosts where *Pentalonia* aphids can proliferate. The epidemiological importance of this aphid as a vector may have far reaching consequences influencing agricultural practices in Hawaii, the Philippines, India and other nations where banana is an economically important staple crop.

Chapter 2: Effect of species, genotype and host plant on the development, reproduction and survival of *Pentalonia* aphids on banana

Abstract

The genus *Pentalonia* (Hemiptera: Aphididae) contains several species that colonize sub-tropical and tropical plants in the order Zingiberales and in the family Araceae. This research compares the life history and developmental parameters of 12 *Pentalonia* lineages originally collected from: taro (*Colocasia* sp.), heliconia (*Heliconia* spp.), red ginger (*Alpinia purpurata*) and banana (*Musa* sp.) on banana leaf discs. Mitochondrial sequences identified 3 and 9 lineages as *Pentalonia nigronervosa* Coquerel and *Pentalonia caladii* van der Goot. Microsatellite analysis separated the lineages into 7 distinct genotypes. The results indicate that aphid fitness was influenced by maternal host plant, aphid species and genotype. *Pentalonia nigronervosa* and *P. caladii* differed significantly in mean development time, total offspring, and longevity, but did not differ in daily fecundity or life span. *Pentalonia caladii* demonstrated a slightly higher rate of increase (r_m) and net reproductive rate (R_o) and required less time to double in population size. Four lineages were further evaluated for colonization of potted banana plants. The lineages displayed differences in colonization ability and preferred feeding locations. Lineages from heliconia and banana preferred to feed on petioles than on leaves, and the lineage from taro hardly colonized banana plants. This research shows that *P. caladii* is able to colonize and reproduce on banana, but the success varies with both genotype and host plant of origin.

Introduction

The banana aphid, *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) is found in most tropical and subtropical regions where banana is grown, and in some greenhouses throughout Europe and North America (Blackman and Eastop, 2006). *Pentalonia nigronervosa* is viviparous, reproducing by obligate parthenogenesis throughout its host range, although researchers in India have reported the occurrence of oviparous (egg laying) morphs (Bhanotar and Ghosh, 1969). *Pentalonia nigronervosa* has been described as an oligophagous species colonizing plants in the families: Araceae (*Colocasia* sp., *Xanthosoma* spp., *Dieffenbachia* spp. and *Caladium* spp.), Musaceae (*Musa* spp.), Zingiberaceae (*Zingiber* spp., *Alpinia* spp., *Hedychium* spp., *Costus* spp. and *Elettaria* spp.), Heliconiaceae (*Heliconia* spp.) and Cannaceae (*Canna* spp.) (Waterhouse, 1987, Blackman and Eastop, 2000). However, more recent studies indicate *P. nigronervosa* displays host preferences (Foottit et al., 2010) and colonizes plants other than banana with difficulty (Bhadra and Agarwala, 2010, Lomerio and Calilung, 1993). *Pentalonia nigronervosa* was previously reported to contain two forms: “typica” and “caladii” (Eastop, 1966). Some researchers have referred to these forms as sub-species (Viswanathan and Regupathy, 1992, Rajan, 1981, Venugopal et al., 1999) although many other authors have not made that distinction.

Padmalatha and Singh (2001, 2002b) found that *Pentalonia* collected from banana and from alternative host plants differed in antennal characteristics and ultimate rostral segments. More recently, Foottit et al (2010) used both morphological and molecular assays to differentiate *Pentalonia* aphids colonizing banana and alternative plants of the family Araceae and Zingiberaceae. Aphids from banana had longer ultimate rostral segments, not overlapping in length with aphids from other host plants (Foottit et al., 2010). In addition, sequences of mitochondrial and nuclear genes separated *Pentalonia* aphids collected from banana and aphids from other host plants (Zingiberaceae, Araceae) into two distinct and well-supported clades corresponding to 2 species: *P. nigronervosa* Coquerel and *P. caladii* van der Goot (Foottit et al., 2010). Most recently a population genetics study in Hawaii determined *P. nigronervosa* and *P. caladii* contain at least 52 and 38 multi-locus genotypes (MLG’s), respectively (Galambao, 2011).

Previous experiments using aphids from the genus *Pentalonia* have focused on one clone or form (Anhalt and Almeida, 2008, Rajan, 1981, Viswanathan and Regupathy, 1992, Robson et al., 2007). Other works did not report the host plant from which aphids were collected, or the number of aphids used to establish the colonies from which biological data were drawn (Rajan, 1981, Padmalatha and Singh, 2002a, Viswanathan and Regupathy, 1992). Recently, Bhadra and Agarwala (2010) used two aphid colonies, one established from banana and one from taro, and compared their performance on the different host plants. When reciprocal transfers were made, aphids from taro were unable to colonize banana effectively (Bhadra and Agarwala, 2010). Research on the ability of other host associated aphid lineages to develop on banana has not been evaluated.

Pentalonia nigronervosa transmits *Banana bunchy top virus* (BBTV), the most destructive viral pathogen of banana plants worldwide (Kumar et al., 2011, Dale et al., 1992). BBTV is transmitted by *P. nigronervosa* in a persistent, circulative manner (Hu et al., 1996, Anhalt and Almeida, 2008, Magee, 1940). *Pentalonia caladii* is suspected to transmit BBTV (Foottit et al., 2010), and is the known vector of several economically important viruses (Venugopal, 1995) including *Cardamom bushy dwarf virus* (CBDV) a new Babuvirus in the family Nanoviridae (Mandal et al., 2004, Tidona and Darai, 2011). In addition, *P. caladii* is a pest of tropical flowers and ornamentals including ginger, heliconia, and taro (Waterhouse, 1987, Blackman and Eastop, 2000) which are often found growing near banana plantations.

Unlike the transmission of non-persistent viruses, it is believed that competent vectors of circulative plant viruses, including BBTV, must show host plant acceptance through prolonged feeding before transmission can occur. Therefore, understanding the potential ability of *Pentalonia* from different host plants, to colonize banana is a prerequisite to understanding their potential role in the epidemiology of BBTV. In this study, I studied whether *Pentalonia* aphids from ginger, heliconia, taro, and banana have equivalent potential to colonize, live on, and reproduce after transfer to banana. I accomplished this by examining the life history and developmental parameters of 12 distinct *Pentalonia* lineages on banana leaf discs and compared fitness parameters across the lineages. The breeding potential and feeding preference of 4 selected lineages on entire potted banana plants were also examined.

Materials and Methods

Aphid source

In spring of 2010, *Pentalonia* aphids were collected as apterous morphs from: banana (*Musa* L.) (Musaceae), red ginger (*Alpinia purpurata* Vieillard) (Zingiberaceae), heliconia, (*Heliconia* spp. L.) (Heliconiaceae) and taro (*Colocasia esculenta* L.) (Araceae) on the island of Oahu (Table 1). I established 3 colonies from each host plant species for a total of 12 colonies (Table 1). I will refer to these colonies as asexual lineages because each colony was started from an individual female. In order to ensure the successful start of each lineage, individual apterous females were placed in Tashiro cages (1967) containing detached leaves of the host plant from which aphids were collected (Plate I). Cages were maintained within a growth chamber with a temperature of 25°C and 12:12 D: L (VWR International, OR). After approximately 1 week, asexually produced offspring were transferred with a small camel's hair paintbrush to potted plants of the same plant species from which aphids were collected (hereafter referred to as maternal host plants). All plants were contained within poly-organza mesh fabric cages, with a mesh size of 36 cm^{-1} (Super Poly Organza, Hyman Hendler and Sons, Los Angeles, CA). The 12 lineages were maintained in a greenhouse with a natural photoperiod and temperature range of 26 ± 5 °C (Plate II). To maintain colonies, aphids were transferred to new plants each month. After a rearing period of 2-5 months, aphids were used in experiments.

Aphid identification

Aphid lineages were identified to species level by using PCR assays and sequencing of a portion of the Cytochrome Oxidase subunit I (COI) gene following procedures described by Foottit et al. (2010). Lineages were further characterized using 9 microsatellite markers (M62, S24, Ago66, S17b, S16b, S23, AF169, AF-4, and AF-1) and were assigned to multi locus genotypes (MLG's) using the software Genotype and Genodive as previously described (Galambao, 2011, Meirmans and Van Tienderen, 2004).

Host plant source

Banana plants (*M. acuminata* cv Williams AAA Cavendish hybrid) were obtained through the Seed Lab - banana tissue culture facility at the University of Hawaiʻi at Mānoa, College of Tropical Agriculture and Human Resources (CTAHR). Taro (*C. esculenta*), heliconia (*H. stricta*), and red ginger (*A. purpurata*) plants were obtained from a nursery. A mixture of soil-less potting mix containing Sunshine Mix 4 (Sun Gro Horticulture Distribution, Vancouver, Canada), vermiculite, and perlite at a ratio of 3:2:1 was used to pot all plants. At first potting plants were supplied with a slow release fertilizer, Osmocote, containing N:P:K at a ratio of 19-6-12; (The Scotts Company, Marysville, OH) and thereafter plants were fertilized on a biweekly basis with Miracle-Gro fertilizer containing N:P:K at a ratio of 24-8-16 (Scotts Miracle-Gro Products, Inc., Marysville, OH). Plants were kept under greenhouse conditions (26 ± 5°C) until space was unavailable, then plants were moved into large outdoor cages (13 x 25 x 14m; Plate II).

Life table and developmental parameters

Thirty adult aphids were collected from each colony and were transferred using a camel hair brush to Petri dishes (55 x 14 mm; Plate III) containing leaf cuttings embedded in 1% agar containing .087% chlorothalonil (Daconil, TechPac, LLC., Atlanta, GA). Aphids were allowed to produce nymphs overnight, thereafter the adults were removed and the young nymphs were allowed to develop into adults. Adults of equal age were transferred into individual cages in a growth chamber at 25°C (12:12 L:D) and were allowed to produce 10-20 1st instar nymphs overnight. The resulting 1st instar nymphs from each lineage were used to establish cohorts of the same age.

Individual nymphs were transferred into cages constructed by slicing a Falcon tube at ~25 mm from the top of the lid. The lid was then filled with 1% agar containing .087% chlorothalonil. A flame sterilized cork borer was used to excise 11 mm diameter leaf discs from the leaf lamina of the most recently unfurled banana leaf (“cigar leaf”). Cages were sealed with Seal View® laboratory film (Plate IV). Observations were conducted daily by observing aphids through the transparent film. Leaf discs were changed every 3-4 days. The life table study was carried out over the course of 6 months in the fall of 2010. Three replicates were performed.

First instar nymphs from each lineage were observed feeding on banana leaf discs from birth until death. I recorded molting, offspring production and survivorship through daily observations of the cohort. I determined the developmental parameters of mean development time, adult longevity, fecundity, life span and total offspring for all 3 replicates (See Appendix 1 for life history definitions). The parameters were compared separately for each treatment using the General Linear Model (GLM) in SAS. Fixed treatments were maternal host plant (red ginger, heliconia, taro, and banana) species (*P. nigronervosa*, *P. caladii*) and MLG's as determined by microsatellite analysis. Replicates were treated as a random factor. Pairwise comparisons were conducted by a Tukey test.

To determine population estimates on banana, I constructed life tables for each cohort following methods established by Carey (1993). The intrinsic rate of natural increase (r_m), the number of offspring produced per unit time, was determined according to Price (1984). I used the standard equations previously used to construct life tables for *P. nigronervosa* (Robson et al., 2007):

$$r_m=\log_e R_o/T \qquad R_o=\sum l_x m_x$$

$$MT=\ln(R_o)/r_m \qquad DT=\ln 2/r_m$$

The net reproductive rate (R_0), the average number of offspring born to the cohort, was determined by obtaining the net maternity ($l_x m_x$) which is the product of the age specific survival (lx) and age specific birth rates of the cohort. Other life history parameters calculated were mean generation time (T), the mean age of reproduction, and doubling time (DT), the time it takes for the population to double in size for each species and genotype (See Appendix 1for complete definitions of life table parameters).

Plant assay

I evaluated the reproductive capacity and preferred feeding sites of 4 lineages: B_4, G_4, H_3, and T_1 (Table 1) on banana plants under greenhouse conditions. Banana plants were used at a growth stage of 9 leaves and were fertilized and maintained as previously described. Ten 4th instars from each colony were released at the base of the cigar leaf. All plants were enclosed within poly-organza mesh fabric cages held by rubber bands around the base of the pot (Plate V). After 30 days, the number of aphids including: nymphs, aptera and alates were recorded at 5 different locations on the entire banana plant (feeding sites). Feeding sites were: cigar leaf, base of pseudostem and the upper, lower leaf surface and petiole of the each leaf (Plate VI). I used 5 plants per treatment. I compared the number of aphids in each feeding site using the General Linear Model (GLM) in SAS. The fixed treatments were lineage (B_4, G_4, H_3, and T_1) and feeding site (cigar leaf, upper leaf surface, lower leaf surface, petiole, and base of pseudostem). Replicates were treated as a random factor. Pairwise comparisons of mean numbers of aphids per feeding sites were conducted using a Tukey test.

Results

Aphid identification

Table 1 reports the identification as species and genotype for the 12 laboratory established lineages. Sequencing of a portion of the COI followed by a blast search in GenBank identified *P. nigronervosa* and *P. caladii* for 3 and 9 lineages, respectively. *Pentalonia nigronervosa* were collected from banana exclusively, whereas *P. caladii* were collected from the alternative host plants (Table 1). Based on 9 microsatellite markers, 7 distinct MLGs were identified using the software program Genotype and Genodive (Table 1; See Appendix 2 for complete polymorphic loci). Two MLGs, P_1 and P_3, were collected from banana, whereas five MLGs: P_2, P_4, P_5, P_6 and P_7 were collected from red ginger, heliconia and taro (Table 1).

Life table and developmental parameters

All of the 12 Pentalonia lineages were capable, to various degrees, of developing and reproducing on banana under laboratory conditions. Results of the population parameters obtained through life table analysis are reported in Table 3. Aphid genotypes originating from taro experienced the highest nymphal mortality rates ranging from 13-22% versus 2-11% of those from banana. Aphid genotypes maternally derived from red ginger and heliconia (P_4 and P_2) had the highest survival rates (Figure 1). Surprisingly, genotypes P_2 and P_4 experienced an extremely low nymphal mortality in the range of 1-9% (Table 3). Genotypes P_6 and P_7 had the shortest generation times and very short doubling times. Genotype P_5 had the smallest r_m (Table 3). Genotype P_2 had the shortest DT and the greatest R_o. Within the last week of life some aphid genotypes (P_1, P_3, and P_7) were able to give birth to several nymphs after a several day-long break in reproductive output (Figure 1). Some aphids produced offspring on the last day of life. When compared to *P. nigronervosa*, *P. caladii* had the lowest nymph mortality, a longer generation time, a higher intrinsic rate of increase, a shorter doubling time and a higher net reproductive rate (Table 3). By day 36, 50% of the original *P. caladii* cohort was still alive, although only 33% of the cohort of *P. nigronervosa* was alive at that time (Figure 2; Table 4,Table 5; see also Appendix 3-9 for complete life tables compiled for each genotype).

When the developmental parameters of the 12 lineages defined by maternal host plant were examined, I observed significant differences in development time, adult longevity, and lifespan (Table 2, $P < .003$). The total number of offspring produced was also significantly affected by the maternal host plant; however, daily fecundity was not (Table 2, $P < .126$). Aphid lineages collected from red ginger produced significantly more offspring, lived longer as adults and had a longer total lifespan than aphid lineages from banana and taro plants (Table 2). Aphids from banana had the shortest lifespan as adults and produced the least number of offspring and this effect was statistically different from aphid lineages collected from red ginger and heliconia plants. Aphids from heliconia required the longest time to develop into adults (Table 2). Aphids from taro plants had the shortest development time and lifespan and these fitness parameters were statistically different for aphids from heliconia.

When fitness parameters of aphid lineages were separated based on species, *P. caladii* displayed slightly higher fitness on banana leaf discs than *P. nigronervosa* for all of the parameters examined (Table 2) except for fecundity ($F_{1,488}$ = .57, *P* = .449) and development time ($F_{1,488}$ = 0, *P* = .971). Developmental parameters for the 7 MLGs are presented in Table 2. Genotype P_7 had a significantly greater fecundity than all others (Table 2, $F_{6, 483}$ = 5.50, $P < .0001$). Genotype P_4, which corresponds to the "red ginger" lineages, generally exhibited greater fitness than all of the other genotypes in total number of offspring produced, lifespan and longevity as adults (Table 2, $P < .0001$). The genotype P_4 produced significantly more offspring than genotypes P_1, P_3 and P_6 and lived significantly longer than genotypes P_3, P_6, and P_7. Genotype P_7 took the longest time to develop into an adult. In terms of adult longevity, P_4 lived significantly longer than P_1, and P_3. Genotypes with the shortest developmental periods from taro and banana also had the highest fecundity and shortest life spans (Table 2).

Colonization of banana plants

The four lineages chosen: B_4, G_4, H_3, and T_1 represented 4 distinct genotypes: P_3, P_2, P_4, P_5, and 2 *Pentalonia* species (Table 1). After 30 days post-introduction, the mean total number of aphids of *P. nigronervosa* from banana was significantly greater than *P. caladii* from taro ($F_{4, 88}$=2.34, P=.079; Figure 4). All lineages colonized banana plants, however the total average number of aphids from taro was extremely low (Figure 4).

Feeding site had a significant effect on aphid distribution, ($F_{4, 688}$ = 18.24, $P < .0001$) over the entire plant, but mean aphids per feeding site at each leaf position (1-11) were not significantly different (unpublished data), therefore leaves and petioles were standardized to represent one leaf/petiole per plant. The 4th leaf position was chosen at random to include in further analysis. When mean aphids per feeding site were compared using only the 4th leaf position, aphids preferred petioles to cigar leaves ($F_{4, 88}$= 2.77, P=.032). When lineages were evaluated individually only aphids from heliconia were significantly greater on petioles than all other leaf sites ($F_{4, 16}$=5.28, P=.007; Figure 3).

Discussion

This study reveals the effect of species, genotype and maternal host plant on the development, reproduction and survival of *Pentalonia* aphids on banana leaf discs and the colonization potential of *Pentalonia* aphids on entire potted banana plants.

Banana leaf lamina was chosen over midrib cuttings due to the logistical constraints of comparing a large number of lineages. In addition, a previous study showed that fitness of an asexual lineage of *P. nigronervosa* was not significantly different when reared on banana leaf lamina than on midrib cuttings (Robson et al., 2007). Under experimental conditions, I observed lower r_m and life history values than previously found for *P. nigronervosa* (Robson et al., 2007). An explanation for the lower overall fitness obtained may be that *P. nigronervosa* prefers to colonize the pseudostem and leaf midribs of banana plants, and that leaf lamina is a sub-optimal source for aphid development. This is validated by observations of aphid colonization of entire potted banana plants where most *P. nigronervosa* aphids colonized the petioles and basal pseudostem, and only a small percentage (less than 20% on average) colonized the leaf lamina. Ginger, heliconia and banana plants belong to the order Zingiberales, which contains many tropical families of flowering plants. In the field, *P. caladii* feeds primarily on the cigar leaf and, when present, the flower bracts of red ginger and heliconia plants where they can produce dense colonies (personal observation). *Pentalonia* lineages from taro primarily colonize the base and sinuses of the petioles of taro plants. The difference in fitness observed by aphids in the leaf disc assay, especially the high mortality of aphids from taro, may be due to the different ability of each lineage to feed specifically on leaf lamina.

Interestingly, many developmental parameters determined in this study differed by the aphid lineage's maternal host plant. Aphids from taro plants exhibited shorter development periods, higher daily fecundity and shorter life spans on banana than aphids from alternative plants. Banana plants may not satisfy the nutritional requirements of *P. caladii* lineages from taro. It is also possible that banana plants possess compounds that deter *P. caladii* feeding. The effect of these compounds may be further magnified when whole plants were used in experiments. In the colonization trial the taro-derived asexual lineage formed colonies 20 fold less abundant than aphids derived from banana plants.

Some of the lineages used in this study differed in body color. Other authors have also reported color differences in *Pentalonia* aphids collected from costus, anthurium, banana, and taro (Padmalatha and Singh, 2001). Although the color of *Pentalonia* aphids has been described as red, brown or black (Blackman and Eastop, 2006) two of the lineages in the present study (T_1 and T_2) from taro were mostly green. First instar nymphs of these lineages were markedly yellowish and turned greenish through their development. These aphid lineages retained their greenish color while developing on banana; therefore body color does not appear to be an effect of the diet. In the pea aphid, *Acyrthosiphon pisum* (Harris), changes in body color have been associated with endosymbiotic bacteria (Tsuchida et al., 2010) and/or lateral transfer of genes from fungi (Fukatsu, 2010, Moran and Jarvik, 2010). The reason for these color differences in *Pentalonia* is currently unknown. Lineages T_1 and T_2 had much lower nymphal mortality, longer development, and were significantly less fecund than the "red" taro lineage T_3.

Under the conditions used in the life table experiments, the lineages of *P. caladii* exhibited slightly higher fitness than those belonging to *P. nigronervosa*. Once confined to whole plants, the lineage of *P. nigronervosa* displayed remarkably higher performance than *P. caladii* lineages, although all lineages were able to colonize banana plants. These findings indicate that *P. caladii* adapted easily to the specific laboratory conditions or that this species display ecological plasticity in the host plant use. This is supported by the observation that under field conditions *P. caladii* colonizes ginger, heliconia and taro and secondarily banana plants (Foottit et al., 2010, Galambao, 2011). Observed differences in fitness between leaf disc and whole plants experiments may also be due to additional factors including: temperature, colony dynamics and feeding behavior.

Temperature affects *P. nigronervosa* biology and transmission efficiency (Robson et al., 2007, Wu and Su, 1990b), but the effects of temperature on *P. caladii* are unknown. Aphids within greenhouses were exposed to more fluctuating environmental changes in temperature and humidity than aphids within the leaf disc assay. *Pentalonia caladii* populations may be more sensitive to high temperature stress than *P. nigronervosa.*

Aphids often display aggregating behavior that can alter resource availability and lead to competition (Hodgson, 2001). Aphids within leaf disc assays were confined to individual cages and therefore not in competition for resources. Colony dynamics such as group size for colony establishment and temperature regime can affect clonal fitness (Michaud et al., 2006). When aphids were transferred to plants for colonization trials ten aphids established each colony. Aphid lineages from banana performed poorly as individuals but when allowed to develop with other members of their colony they showed the greatest increase in fitness. Aggregate feeding leads to "resource allocation" that may enable those aphids to increase development and fecundity on a particular host (Goggin, 2007, Michaud et al., 2006). Colony behavior such as aggregating may also affect reproductive capacity, therefore only measuring individual aphid fitness and not accounting for colony effects may lead to conserved estimates of fecundity (Hodgson, 2001). Some lineages that poorly performed on banana plants, may have been subjected to colony effects such as alarm pheromone production that may increase the mobility of aphids (Davis and Radcliffe, 2008). Colony behavior can also affect individual performance. When handling aphids on entire plants *P. caladii* retracted stylets faster, walked away faster and had a greater tendency to drop from the plant than *P. nigronervosa* after contact with a brush. Conversely, transferring *P. nigronervosa* required extensive prodding to promote retraction of the stylets from banana plant tissues.

Feeding behavior such as how long it takes for the aphid to explore a plant surface, reach the phloem tissue and the quantity of sap that can be ingested may affect successful colonization. *Pentalonia caladii* from red ginger and heliconia were able to colonize entire potted banana plants over a 30 day period; therefore it does not seem likely they had trouble feeding on phloem tissues. Aphids collected from taro colonized banana plants, but persisted at low numbers. Colonization success could be due to differences in penetration of sieve elements. Electronic penetration studies are effective at determining the amount of time aphids feed on phloem tissues and the time required for reaching those tissues (Girm et al., 1992). It would be valuable to examine aphid feeding behavior of *Pentalonia* lineages using the EPG technique, especially to understand the mechanism behind the poor colonization success of *P. caladii* from taro. Evaluating evolutionary phylogenetic relationships may also shed light on why some aphid lineages from taro exhibited poor fitness and colonization potential on banana.

The results herein increase our knowledge about *Pentalonia* species and their potential ability to colonize banana. All *Pentalonia* tested were able to live on banana leaf lamina and displayed similar fitness irrespective of the maternal host. However, once on potted banana plants some of those lineages markedly differed in colonization success. This research shows *P. caladii* from red ginger, heliconia and taro have the potential to colonize banana in the field. Further research is warranted to assess the epidemiological role of *P. caladii* in the spread of BBTV.

Tables

Table 1. *Pentalonia* aphid collection and identification; host plant, locale, species and MLG profile

Colony	Host Plant	Collection Date	GPS Coordinates	Species*	MLG
B1	Banana (*Musa* sp.)	2/12/2010	N21 22.549 W157 55.605	*P. nigronervosa*	P1
B3	Banana (*Musa* sp.)	2/17/2010	N21 31.678 W158 02.275	*P. nigronervosa*	P3
B4†	Banana (*Musa* sp.)	4/7/2010	N21 16.995 W157 42.025	*P. nigronervosa*	P3
G1	Ginger (*Alpinia* sp.)	5/7/2010	N21 18.883 W157 48.422	*P. caladii*	P4
G3	Ginger (*Alpinia* sp.)	5/7/2010	N21 16.358 W157 49.354	*P. caladii*	P4
G4†	Ginger (*Alpinia* sp.)	5/7/2010	N21 16.565 W157 45.467	*P. caladii*	P4
H1	Heliconia (*Heliconia* sp.)	5/7/2010	N21 18.509 W157 48.368	*P. caladii*	P2
H2	Heliconia (*Heliconia* sp.)	5/7/2010	N21 18.944 W157 48.383	*P. caladii*	P4
H3†	Heliconia (*Heliconia* sp.)	5/7/2010	N21 19.033 W157 49.919	*P. caladii*	P2
T1†	Taro (*Colocasia* sp.)	5/7/2010	N21 18.882 W157 48.421	*P. caladii*	P5
T2	Taro (*Colocasia* sp.)	5/7/2010	N21 17.155 W157 40.417	*P. caladii*	P6
T3	Taro (*Colocasia* sp.)	5/7/2010	N21 20.235 W157 43.317	*P. caladii*	P7

*Species identification based on Mitochondrial COI sequences
**MLG designated by 9 microsatellite markers followed by an assessment using Genotype and Genodive software by Marciana Galambao
†Aphid lineages evaluated for colonization success on entire potted banana plants

Table 2. Mean ± SE (range) of developmental parameters of *P. nigronervosa* and *P. caladii* (by colony, species and genotype) when reared on banana leaf discs at 25°C. Means followed by different letters indicate significant differences.

		Fecundity (no.)	Total Offspring (d)	Lifespan (d)	Development time (d)	Adult longevity (d)
Maternal Host Plant H Host	Banana	1.10a ± 0.04 (.55-2.18)	20.78c ± .64 (6-39)	29.78b ± .81 (2-50)	12.25ab ± .12 (10-15)	19.70c ± .55 (7-36)
	Ginger	1.05a ± .03 (.58-2.50)	24.43a ± .61 (4-43)	35.55a ± .78 (6-51)	12.35b ± .10 (10-15)	24.35a ± .62 (2-38)
	Heliconia	1.09a ± .04 (.50-2.56)	23.64ab ± .90 (2-47)	32.77a ± .99 (3-51)	12.53a ± .13 (10-16)	22.33ab ± .75 (2-36)
	Taro	1.18a ± .04 (.50-3.00)	22.61bc ± .80 (1-46)	29.08b ± .97 (3-47)	11.98b ± .10 (10-16)	20.56bc ± .69 (1-33)
	df	3, 486	3, 486	3, 532	3, 486	3, 486
	F	1.92	9.03	10.67	4.82	8.87
	P	0.126	<.0001	<.0001	0.003	<.0001
Species	*nigronervosa*	1.10a ± .04 (.55-2.18)	20.78b ± .65 (6-39)	29.78b ± .81 (2-50)	12.25a ± .12 (10-15)	19.71b ± .55 (7-36)
	caladii	1.11a ± .02 (.50-3.00)	23.53a ± .44 (1-47)	32.40a ± .55 (3-51)	12.28a ± .06 (10-16)	22.47a ± .40 (1-38)
	df	1, 488	1, 488	1, 534	1, 488	1, 488
	F	.57	16.16	14.42	0	14.90
	P	.449	<.0001	<.0001	.971	<.0001

Table 2. (Continued) Mean ± SE (range) of developmental parameters of *P. nigronervosa* and *P. caladii* (by colony, species and genotype) when reared on banana leaf discs at 25°C. Means followed by different letters indicate significant differences.

		Fecundity (no.)	Total Offspring (d)	Lifespan (d)	Development time (d)	Adult longevity (d)
Genotype						
	P1	1.08b ± .06 (.63-1.92)	20.57b ± 1.00 (9-34)	30.28abc ± 1.21 (10-45)	12.29ab ± 0.21 (10-15)	19.79b ± .84 (8-33)
	P2	1.11b ± .05 (.50-2.56)	24.06ab ± 1.10 (2-47)	32.53ab ± 1.26 (3-47)	12.38a ± 0.16 (10-15)	22.33ab ± .96 (2-35)
	P3	1.11b ± .47 (.55-2.18)	20.88b ± 0.83 (6-39)	29.51bc ± 1.06 (2-50)	12.24ab ± 0.14 (10-15)	19.66b ± 0.71 (7-36)
	P4	1.06b ± .03 (.58-2.50)	24.10a ± 0.59 (2-47)	35.00a ± 0.71 (4-51)	12.45a ± 0.09 (10-16)	23.89a ± 0.56 (2-38)
	P5	1.12b ± .05 (.68-2.00)	24.26ab ± 1.55 (8-46)	30.38abc ± 1.74 (3-44)	12.33a ± 0.17 (11-16)	21.83ab ± 1.05 (8-32)
	P6	1.05b ± .06 (.50-2.40)	20.51b ± 1.17 (1-42)	29.96bc ± 1.66 (3-45)	12.05ab ± 0.14 (11-15)	20.70ab ± 1.24 (1-33)
	P7	1.38a ± .09 (.70-3.00)	23.10ab ± 1.37 (4-43)	27.00c ± 1.66 (4-47)	11.57b ± 0.18 (10-14)	19.14ab ± 1.25 (2-33)
	df	6, 483	6, 483	6, 529	6, 483	6, 483
	F	5.50	4.85	5.65	2.93	4.57
	P	<.0001	<.0001	<.0001	=0.008	<.0001

Table 3. Life history parameters obtained through complete life table analysis of *Pentalonia* aphids (species and genotype) when reared on banana leaf discs at 25°C.

Colony ID		Nymph Mortality	Generation time (T)	Intrinsic rate of natural increase (r_m)	Doubling time (DT)	Net Reproductive rate (R_o)
MLG	P_1	0.11	21.29	0.129	5.38	15.57
	P_2	0.02	23.16	0.130	5.34	20.25
	P_3	0.10	21.83	0.129	5.39	16.58
	P_4	0.02	23.32	0.131	5.27	21.44
	P_5	0.17	22.03	0.125	5.53	15.81
	P_6	0.13	20.93	0.134	5.18	16.42
	P_7	0.23	20.52	0.133	5.21	15.38
Species	*nigronervosa*	0.11	21.65	0.129	5.39	16.22
	caladii	0.11	22.67	0.131	5.31	19.28

Table 4. Complete life table for *P. nigronervosa* on banana leaf discs at 25°C.

Age Class, x	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
0	1.000	1.000	0.000	0.000	1.000	30.780	30.780	100
1	1.000	0.990	0.010	0.010	0.995	29.780	29.780	100
2	0.990	1.000	0.000	0.000	0.990	28.785	29.076	99
3	0.990	0.990	0.010	0.010	0.985	27.795	28.076	99
4	0.980	0.969	0.031	0.030	0.965	26.810	27.357	98
5	0.950	1.000	0.000	0.000	0.950	25.845	27.205	95
6	0.950	1.000	0.000	0.000	0.950	24.895	26.205	95
7	0.950	1.000	0.000	0.000	0.950	23.945	25.205	95
8	0.950	1.000	0.000	0.000	0.950	22.995	24.205	95
9	0.950	0.979	0.021	0.020	0.940	22.045	23.205	95
10	0.930	1.000	0.000	0.000	0.930	21.105	22.694	93
11	0.930	0.989	0.011	0.010	0.925	20.175	21.694	93
12	0.920	1.000	0.000	0.000	0.920	19.250	20.924	92
13	0.920	0.989	0.011	0.010	0.915	18.330	19.924	92
14	0.910	1.000	0.000	0.000	0.910	17.415	19.137	91
15	0.910	1.000	0.000	0.000	0.910	16.505	18.137	91
16	0.910	1.000	0.000	0.000	0.910	15.595	17.137	91
17	0.910	1.000	0.000	0.000	0.910	14.685	16.137	91
18	0.910	1.000	0.000	0.000	0.910	13.775	15.137	91
19	0.910	0.989	0.011	0.010	0.905	12.865	14.137	91
20	0.900	0.989	0.011	0.010	0.895	11.960	13.289	90
21	0.890	1.000	0.000	0.000	0.890	11.065	12.433	89
22	0.890	0.978	0.022	0.020	0.880	10.175	11.433	89
23	0.870	0.977	0.023	0.020	0.860	9.295	10.684	87
24	0.850	0.965	0.035	0.030	0.835	8.435	9.924	85
25	0.820	0.927	0.073	0.060	0.790	7.600	9.268	82
26	0.760	0.974	0.026	0.020	0.750	6.810	8.961	76
27	0.740	0.892	0.108	0.080	0.700	6.060	8.189	74
28	0.660	0.939	0.061	0.040	0.640	5.360	8.121	66
29	0.620	0.984	0.016	0.010	0.615	4.720	7.613	62
30	0.610	0.918	0.082	0.050	0.585	4.105	6.730	61
31	0.560	0.875	0.125	0.070	0.525	3.520	6.286	56
32	0.490	0.939	0.061	0.030	0.475	2.995	6.112	49
33	0.460	0.870	0.130	0.060	0.430	2.520	5.478	46
34	0.400	0.950	0.050	0.020	0.390	2.090	5.225	40
35	0.380	0.868	0.132	0.050	0.355	1.700	4.474	38
36	0.330	0.818	0.182	0.060	0.300	1.345	4.076	33
37	0.270	0.778	0.222	0.060	0.240	1.045	3.870	27
38	0.210	0.714	0.286	0.060	0.180	0.805	3.833	21

Table 4. (Continued) Complete life table for *P. nigronervosa* on banana leaf discs at 25°C.

Age Class, x	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
39	0.150	0.867	0.133	0.020	0.140	0.625	4.167	15
40	0.130	0.923	0.077	0.010	0.125	0.485	3.731	13
41	0.120	0.917	0.083	0.010	0.115	0.360	3.000	12
42	0.110	0.909	0.091	0.010	0.105	0.245	2.227	11
43	0.100	0.400	0.600	0.060	0.070	0.140	1.400	10
44	0.040	0.250	0.750	0.030	0.025	0.070	1.750	4
45	0.010	1.000	0.000	0.000	0.010	0.045	4.500	1
46	0.010	1.000	0.000	0.000	0.010	0.035	3.500	1
47	0.010	1.000	0.000	0.000	0.010	0.025	2.500	1
48	0.010	1.000	0.000	0.000	0.010	0.015	1.500	1
49	0.010	0.000	1.000	0.010	0.005	0.005	0.500	1
50	0.000			0.000	0.000	0.000		

Table 5. Complete life table for *P. caladii* on banana leaf discs at 25°C.

Age Class, x	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
0	1.000	1.000	0.000	0.000	1.000	33.737	33.737	304
1	1.000	1.000	0.000	0.000	1.000	32.737	32.737	304
2	1.000	0.990	0.010	0.010	0.995	31.737	31.737	304
3	0.990	0.983	0.017	0.016	0.982	30.742	31.048	301
4	0.974	0.990	0.010	0.010	0.969	29.760	30.564	296
5	0.964	0.993	0.007	0.007	0.961	28.791	29.872	293
6	0.957	0.993	0.007	0.007	0.954	27.831	29.074	291
7	0.951	0.990	0.010	0.010	0.946	26.877	28.272	289
8	0.941	0.997	0.003	0.003	0.939	25.931	27.563	286
9	0.938	0.996	0.004	0.003	0.936	24.992	26.658	285
10	0.934	1.000	0.000	0.000	0.934	24.056	25.750	284
11	0.934	0.996	0.004	0.003	0.933	23.122	24.750	284
12	0.931	0.996	0.004	0.003	0.929	22.189	23.836	283
13	0.928	0.996	0.004	0.003	0.926	21.260	22.918	282
14	0.924	0.996	0.004	0.003	0.923	20.334	21.998	281
15	0.921	0.996	0.004	0.003	0.919	19.411	21.075	280
16	0.918	1.000	0.000	0.000	0.918	18.492	20.149	279
17	0.918	1.000	0.000	0.000	0.918	17.574	19.149	279
18	0.918	1.000	0.000	0.000	0.918	16.656	18.149	279
19	0.918	0.989	0.011	0.010	0.913	15.738	17.149	279
20	0.908	0.993	0.007	0.007	0.905	14.826	16.330	276
21	0.901	0.993	0.007	0.007	0.898	13.921	15.445	274
22	0.895	0.989	0.011	0.010	0.890	13.023	14.555	272
23	0.885	0.978	0.022	0.020	0.875	12.133	13.712	269
24	0.865	0.970	0.030	0.026	0.852	11.258	13.013	263
25	0.839	0.980	0.020	0.016	0.831	10.406	12.406	255
26	0.822	0.976	0.024	0.020	0.813	9.576	11.644	250
27	0.803	0.992	0.008	0.007	0.799	8.763	10.918	244
28	0.796	0.955	0.045	0.036	0.778	7.964	10.004	242
29	0.760	0.961	0.039	0.030	0.745	7.186	9.457	231
30	0.730	0.959	0.041	0.030	0.715	6.441	8.820	222
31	0.701	0.948	0.052	0.036	0.683	5.725	8.171	213
32	0.664	0.955	0.045	0.030	0.650	5.043	7.589	202
33	0.635	0.938	0.062	0.039	0.615	4.393	6.920	193
34	0.595	0.906	0.094	0.056	0.567	3.778	6.345	181
35	0.539	0.939	0.061	0.033	0.523	3.211	5.951	164
36	0.507	0.909	0.091	0.046	0.484	2.688	5.305	154
37	0.461	0.907	0.093	0.043	0.439	2.204	4.786	140

Table 5. (Continued) Complete life table for P. caladii on banana leaf discs at 25°C.

Age Class, x	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
38	0.418	0.827	0.173	0.072	0.382	1.765	4.224	127
39	0.345	0.771	0.229	0.079	0.306	1.383	4.005	105
40	0.266	0.926	0.074	0.020	0.257	1.077	4.043	81
41	0.247	0.787	0.213	0.053	0.220	0.821	3.327	75
42	0.194	0.814	0.186	0.036	0.176	0.600	3.093	59
43	0.158	0.813	0.188	0.030	0.143	0.424	2.688	48
44	0.128	0.641	0.359	0.046	0.105	0.281	2.192	39
45	0.082	0.680	0.320	0.026	0.069	0.176	2.140	25
46	0.056	0.588	0.412	0.023	0.044	0.107	1.912	17
47	0.033	0.800	0.200	0.007	0.030	0.063	1.900	10
48	0.026	0.500	0.500	0.013	0.020	0.033	1.250	8
49	0.013	0.500	0.500	0.007	0.010	0.013	1.000	4
50	0.007	0.000	1.000	0.007	0.003	0.003	0.500	2
51	0.000			0.000	0.000	0.000		0

Plates

Plate I. Self watering acrylic Tashiro cages used to establish each *Pentalonia* asexual lineage (left) and close up of aphid rearing arena (right).

Plate II. *Pentalonia* aphid colony rearing under greenhouse conditions (left) and outdoor cages used to grow insect free large potted banana plants (right). The leaf lamina of these plants was used to rear all *Pentalonia* lineages throughout the life table study.

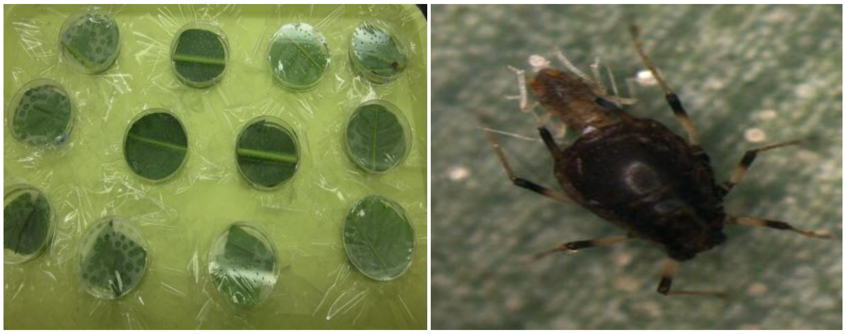

Plate III. Petri dishes used to rear aphid lineages (collected from greenhouse colonies) two generations at 25°C to ensure same age cohorts (left) and a close up of *P. nigronervosa* depositing a 1st instar nymph onto banana leaf lamina (right).

Plates (Continued)

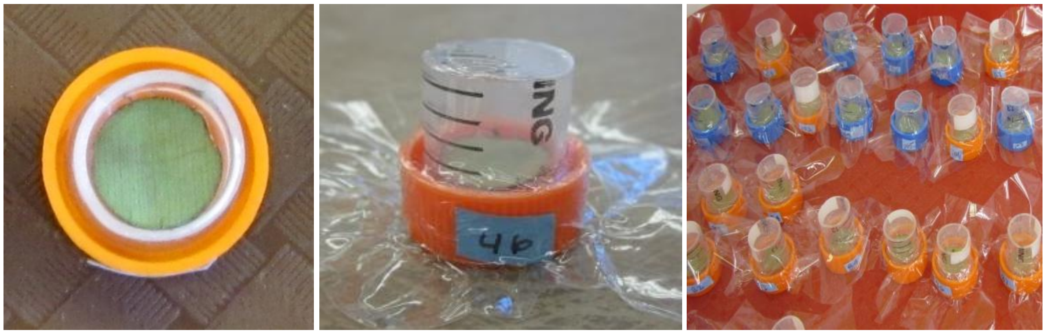

Plate IV. Cage used to observe individual aphids (belonging to a cohort) from birth until death for age specific molting, reproduction and survival during the life table study.

Plate V. Methods for each plant/lineage combination in the colonization study. Ten 4th instars were released at the base of the cigar leaf. Each plant was caged and left in a greenhouse for 30 days.

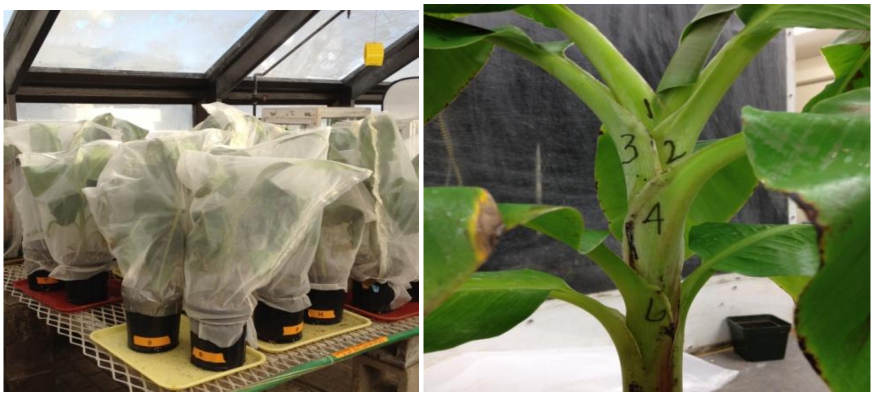

Plate VI. Methods for each plant/lineage combination in the colonization study. Place caged plants in greenhouse. After 30 days a number from 1-11 for each leaf sheath (cigar leaf=0) was assigned to each plant and aphids were counted at the five feeding sites (cigar leaf, basal pseudostem, and upper and lower leaf surface and petiole) at each leaf number.

Plates (Continued)

Plate VII. Systematic removal of leaf sheaths for colonization study to count aphids for each leaf position (1-11) at each feeding site (cigar leaf, basal pseudostem, and upper and lower leaf surface and petiole).

Figures

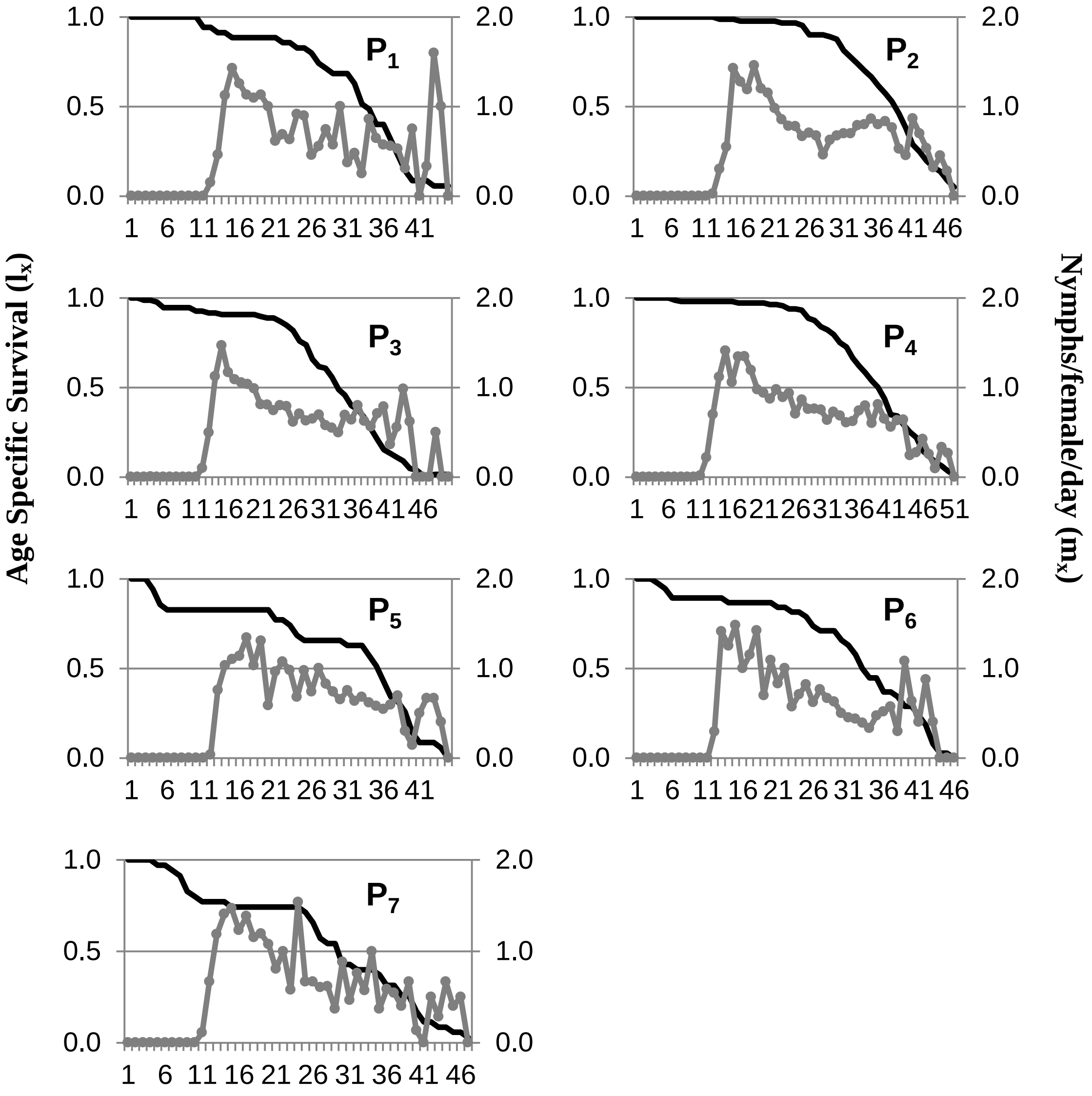

Figure 1. Age Specific Survival (lx) (primary axis; smooth line) and Age Specific Fecundity (mx) (secondary axis; dotted line) as assessed through life table analysis for 2 MLG's (P1, P3) of P. nigronervosa and 5 MLG's (P2, P4, P5, P6, P7) of P. caladii reared on banana leaf discs at 25°C.

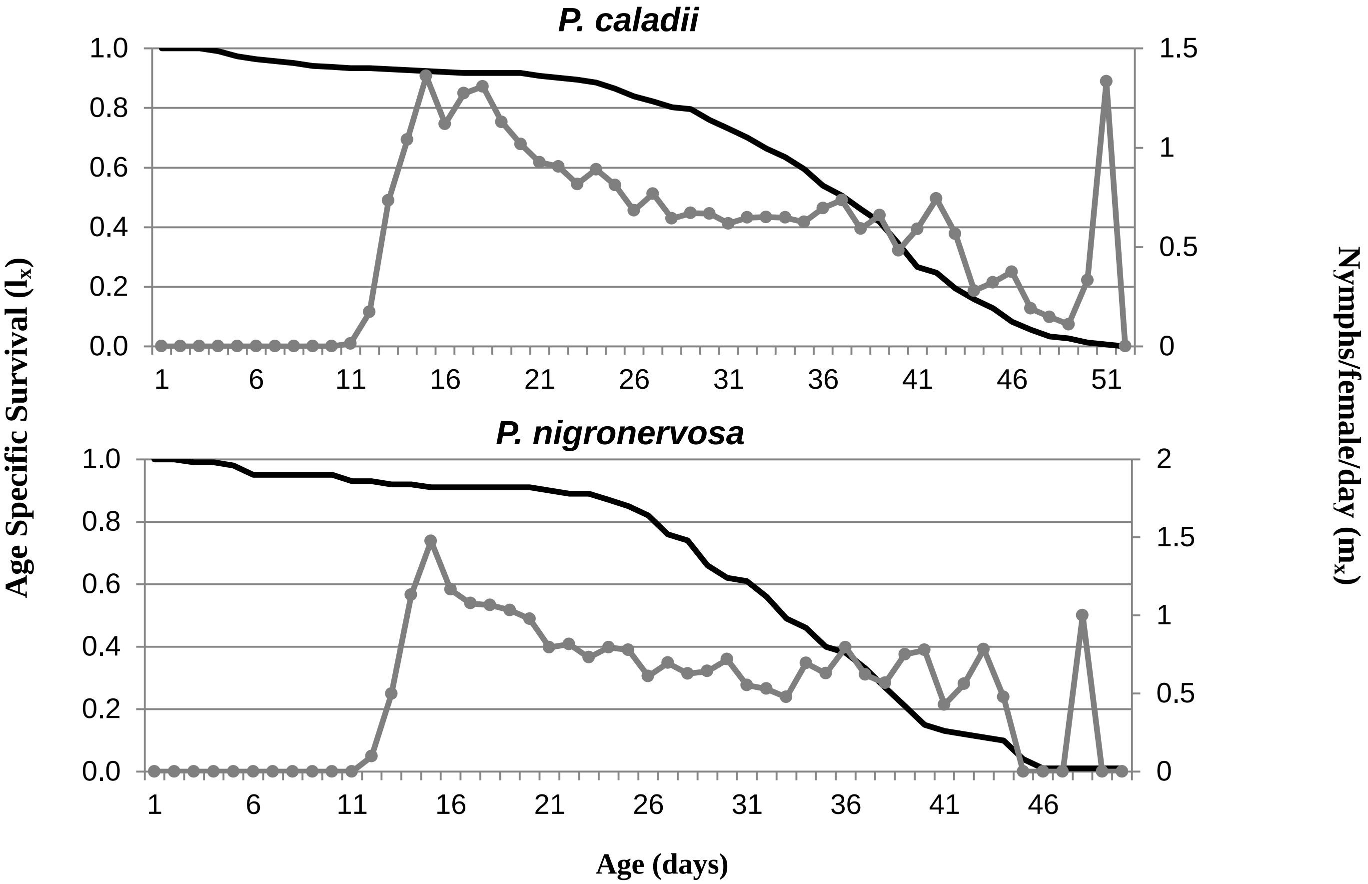

Figure 2. Age Specific Survival (lx) (primary axis; smooth line) and Age Specific Fecundity (mx) (secondary axis; dotted line) of *P. nigronervosa* and *P. caladii* obtained through life table analysis on banana leaf discs at 25°C.

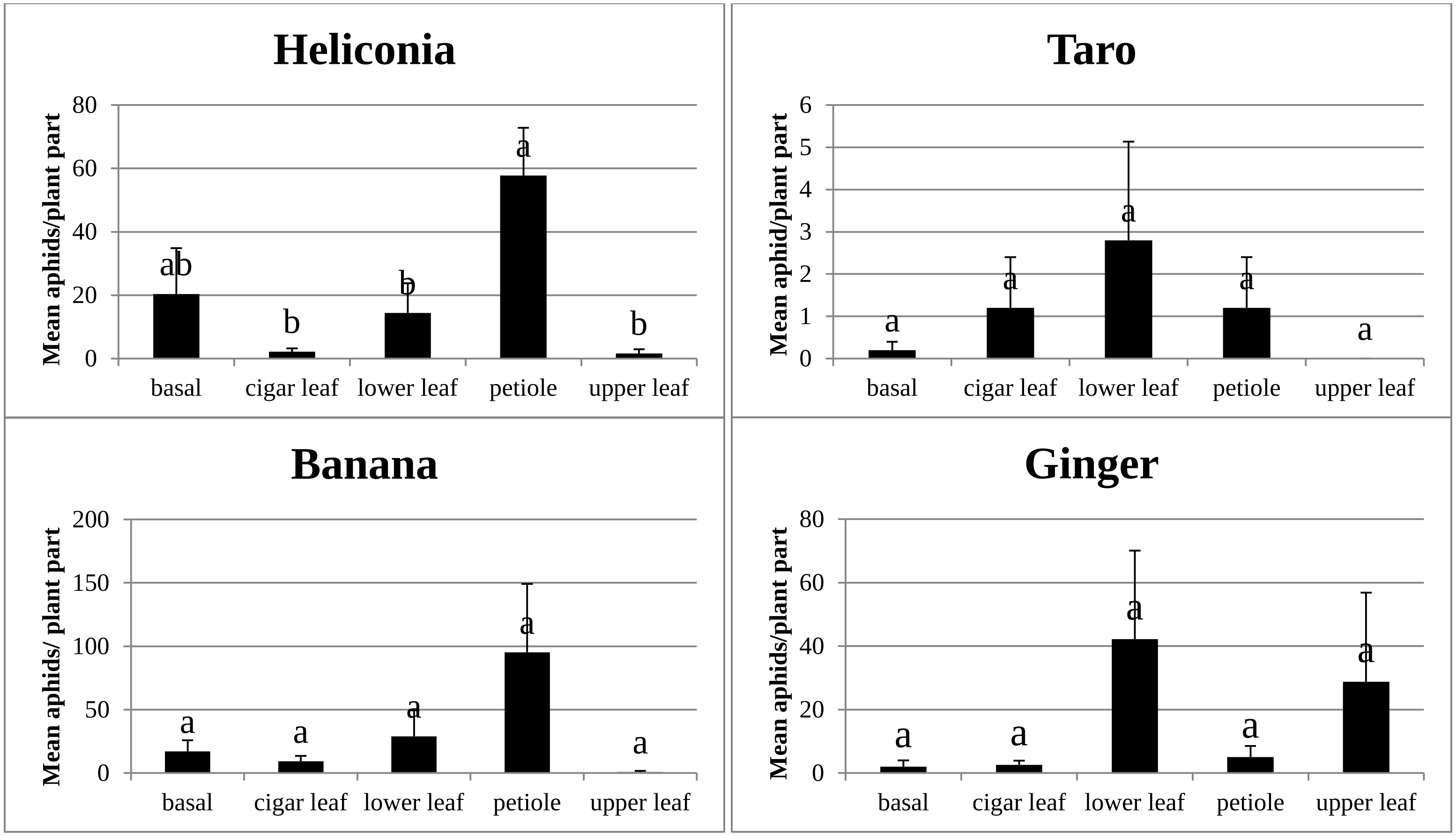

Figure 3. Mean ± SE aphids per feeding site for each asexual lineage (B4, G4, H3, T1) on entire potted banana plants. Horizontal axis indicates original host plants that lineages were collected from. Aphids were counted at the base of the pseudostem, the cigar leaf and on the 4th leaf blade (upper and lower leaf surface) and petiole. Letters indicate significant differences.

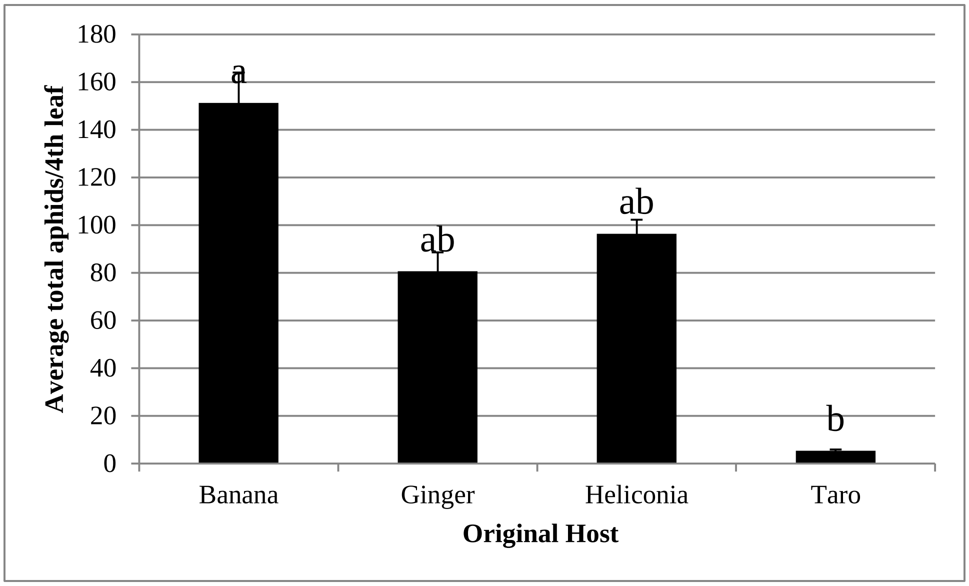

Figure 4. Average total aphids per lineage 30 days post-release of ten 4th instar *Pentalonia* aphids at the base of the cigar leaf of potted banana plants.

Chapter 3: Transmission of *Banana bunchy top virus* by asexual lineages of aphids from the genus *Pentalonia* (Hemiptera: Aphididae)

Abstract

Banana bunchy top virus (BBTV) is the most destructive viral disease of banana plants worldwide. The virus is spread by the banana aphid, *Pentalonia nigronervosa* Coquerel. *Pentalonia caladii* van der Goot, the cardamom aphid, is a closely related species of the banana aphid. In the past, *P. caladii* has been regarded as a sub-species or a form of *P. nigronervosa*, therefore, earlier studies may not have accurately determined the transmission capabilities of each species. In this work, I tested the transmission of BBTV by 12 laboratory reared asexual lineages of *Pentalonia* aphids derived from 4 different host plants: taro, heliconia, red ginger, and banana. Mitochondrial sequences identified 3 and 9 lineages as *P. nigronervosa* and *P. caladii.* Microsatellite analysis separated the lineages into 7 distinct genotypes. A leaf disc assay was devised to perform transmission experiments; fourth instar nymphs were allowed to acquire BBTV from infected banana leaves during a 4-day acquisition access period (AAP). Aphids were then transferred to healthy leaf discs for a 2-day inoculation access period (IAP). Furthermore, four lineages were selected to perform transmission assays using banana plantlets. In leaf disc assays, all lineages were able to vector BBTV with no significant differences between species or genotype. However, when plantlets were used, *P. caladii* derived from red ginger and heliconia plants failed to transmit BBTV.

Introduction

Banana (Musaceae: *Musa* sp.) is cultivated in more than 120 countries and is an important staple crop to millions of people (Jones, 2000). Hawaii leads the United States in fresh market production of banana and banana is the state's 3rd largest fruit crop (NASS, 2010). Banana bunchy top disease (BBTD; Plate VIII) caused by *Banana Bunchy top virus* (BBTV) is one of the most important constraints to crop production in Hawaii, and in several other countries of Asia, Africa, and the Pacific (Dale et al., 1992, Robson et al., 2007, Kumar et al., 2011).

BBTV was first recorded from Fiji in 1889, although the virus is believed to have originated in South Asia (Magee, 1940). In Hawaii, BBTV was first reported in 1989 on the Island of Oahu (Conant, 1992) and has spread to Hawaii (1995), Kauai (1997), Maui (2002) and Molokai (2005) (Nelson, 2006, Kepler and Rust, 2011) most likely through the movement of infected plant material (Almeida et al., 2009). BBTV is an ssDNA Babuvirus belonging to the family Nanoviridae. BBTV is made up of 6 circular DNA components (Fauquet et al., 2005) of approximately 1000 nucleotides each. Each genomic component is separately encapsidated within non-enveloped icosahedral particles of 18-20 nm in diameter (Wu and Su, 1990a, Xie and Hu, 1995). Typical bunchy top symptoms include: chlorosis of leaf margins, narrowing and bunching of successive leaves, Morse code dashes, hooking along the midrib of the leaves, and dark green streaking of petioles (Thomas et al., 1994, Dale et al., 1992). BBTV can move systemically to infect the entire banana corm and new shoots derived from it (Dale and Harding, 1998). In the field, symptoms are expressed 25-85 days after the initial infection of BBTV (Hooks et al., 2008).

BBTV is spread through aphid transmission and through planting of infected plant material (Dale et al., 1992, Magee, 1927). To date, *Pentalonia nigronervosa* Coquerel, the banana aphid, is the only known vector. BBTV is transmitted in a persistent circulative manner and adult aphids appear to be more efficient vectors than 3rd instar nymphs (Anhalt and Almeida, 2008). BBTV is retained in the vector after molting and no transovarial transmission has been reported (Magee, 1927). BBTV may be acquired and transmitted within a minimum acquisition access period (AAP) and inoculation access period (IAP) of 4 h and 15 min, respectively (Hu et al., 1993). There is a detectable latent period for transmission in the aphid estimated at 20-28 h (Anhalt and Almeida, 2008).

Pentalonia nigronervosa was previously reported to contain two forms: "typica" and "caladii" (Eastop, 1966) which have recently been elevated to full species (Foottit et al., 2010). Foottit et al (2010) has used both morphological and molecular methods to differentiate *Pentalonia* aphids colonizing banana and alternative plants of the families Araceae and Zingiberaceae. With the taxonomic split of the banana aphid into *P. nigronervosa* and *P. caladii,* it is unclear if both species are competent vectors of BBTV.

Pentalonia caladii is a known vector of several economically important viruses (Venugopal, 1995) including *Cardamom bushy dwarf virus* (CBDV) a new Babuvirus within the family Nanoviridae (Mandal et al., 2004, Tidona and Darai, 2011). In addition, *P. caladii* is a pest of tropical flowers and ornamentals including ginger, heliconia and taro (Waterhouse, 1987, Blackman and Eastop, 2000). In Hawaii, those plants often grow in close proximity to banana fields.

Persistent circulative viruses, for example those belonging to the family Luteoviridae, are generally transmitted with a high degree of specificity (Gray and Gildow, 2003). Also, because aphids are known to evolve host plant races, it is possible that certain aphid genotypes may be better adapted to transmit plant viruses than others (Symmes and Perring, 2007). For instance, the greenbug, *Schizaphis graminum*, the bird cherry-oat aphid, *Rhopalosiphum padi* and the English grain aphid, *Sitobion avenae* show intra-specific variation in vector transmission efficiency of *Barley yellow dwarf virus* BYDV-MAV or PAV isolates (Bencharki et al., 2000, El-Yamani and Bencharki, 1997, Dedryver et al., 2005, Sadeghi et al., 1997). Recently, a population genetic study of *Pentalonia* aphids conducted in Hawaii found that *P. nigronervosa* and *P. caladii* included 52 and 38 distinct genotypes, respectively, and that these species displayed host plant associations (Galambao, 2011). Transmission efficiency across aphid clonal lineages may be related to differences in fitness and host plant association (Dedryver et al., 2005) It is currently unknown if distinct genotypes of *Pentalonia* spp. exhibit variations in transmission competency of BBTV.

In a previous work, I characterized the life history of 12 asexual lineages of *Pentalonia* aphids on banana (Chapter 2). The fitness parameters on banana were segregated according to species (*P. nigronervosa*, *P. caladii*), genotype, and maternal host plant (banana, red ginger, heliconia, and taro) (see Chapter 2; Table 1). Many life history parameters showed variation at species, genotype and maternal host plant level (Chapter 2; Table 2, Table 3). In this chapter, I performed transmission assays using both leaf discs and banana plantlets to determine the effect of species, genotype and original host plant on the transmission of BBTV.

Material and Methods

Aphid collection and rearing

In the spring of 2010, *Pentalonia* aphids were collected from 4 host plants: banana (*Musa* sp.), red ginger (*Alpinia purpurata)*, heliconia (*Heliconia* sp.) and taro (*Colocasia esculenta)* on the island of Oahu, Hawaii (Chapter 2; Table 1). To establish each asexual lineage, one apterous female was placed on a plant host leaf contained within a self-watering acrylic cage (Tashiro, 1967). Tashiro cages were maintained for one week in a growth chamber (25°C and 12/12 DL: VWR International,OR)(Chapter 2; Plate I) where aphids reproduced through apomictic parthenogenesis. Progeny were then reared under greenhouse conditions (26 ± 5 °C) on their original field host. I established three colonies from each host plant species for a total of 12 colonies (Chapter 2; Table 1). All plants were contained within poly-organza mesh fabric cages (Super Poly Organza, Hyman Hendler and Sons, Los Angeles, CA). The colonies were renewed by transferring aphid-infested leaves to new plants. After a rearing period of 9-12 months, aphids were used in experiments.

Aphid identification

Aphid lineages were identified as species by sequencing of a portion of the gene, Cytochrome Oxidase Sub-unit I (COI) following procedures described by Foottit et al. (2010). Asexual lineages were characterized at genotypic level using 9 microsatellite markers (M62, S24, Ago66, S17b, S16b, S23, AF169, AF-4, and AF-1) and were assigned to multi locus genotypes (MLG's) using the software Genotype and Genodive as previously described (Meirmans and Tienderen, 2004, Galambao, 2011) (Appendix 2).

Plants

Banana plants (*Musa acuminata*; cv Williams) were generated from tissue culture by the Seed Lab of the University of Hawai'i at Mānoa, College of Tropical Agriculture and Human Resources. Heliconia (*Heliconia stricta),* taro (*Colocasia esculenta*) and red ginger (*Alpinia purpurata)* plants were obtained from a nursery. Potting media contained Sunshine Mix 4 (Sun Gro Horticulture Distribution, Vancouver, Canada), vermiculite and perlite at ratio of 3: 2: 1. At first, potted plants were provided with Osmocote, a slow release fertilizer containing a ratio of 19-6-12 N: P : K (The Scotts Company, Marysville, OH), thereafter plants were fertilized biweekly with Miracle-Gro containing a ratio of 24-8-16 N:P:K (Scotts Miracle-Gro Products, Inc., Marysville, OH). Plants were grown under greenhouse conditions with natural light and temperature range of 26 ± 5°C. Alternatively, large plants were grown in outdoor cages (45 x 84 x 47 cm) (Chapter 2; Plate II).

Transmission assays were accomplished using an isolate of BBTV used in previous experiments. The BBTV isolate was collected on the island of Oahu and was maintained within potted banana plants within a greenhouse (Bressan and Watanabe, 2011). Every month, BBTV was inoculated using viruliferous aphids to five healthy banana plants at the growing stage of 9 leaves. Aphids were allowed to feed for a 1 week IAP and were then sprayed with 2% potassium salts of fatty acids (Safer insecticidal soap, Woodstream Corporation, Lilitz, PA). One month post inoculation, plants that started showing symptoms were pruned approximately 5 cm above the basal pseudostem. The pruned plants that produced new infected shoots expressing severe symptoms were used to supply inoculums for the transmission assays. BBTV plants were fertilized following the same procedures outlined above.

Leaf disc transmission assay

Fourth instar aphids collected from the rearing plants were starved for approximately 1 hour and were then allowed a 4 day AAP on excised BBTV infected leaves which were embedded in 1% agar within Petri dishes (55 x 14 mm; Plate IX). Thereafter, groups of three aphids were transferred to inoculation cages containing healthy banana leaf discs for an IAP of two days (Plate X). Inoculation cages were constructed from 15 ml falcon tubes by slicing the tubes 2.5 mm from the lid. The lid was filled with 1% agar containing .087% Chlorothalonil (Daconil, TechPac, LLC, Atlanta, GA). A flame-sterilized cork borer was used to excise 11 mm leaf discs from healthy lamina of the most recently unfurled banana leaf ("cigar leaf"). The cages were sealed with Parafilm and perforated using an insect pin to avoid condensation (Plate X). Leaf disc transmission assays took place in a growth chamber at 25°C with a 12: 12 L: D photoperiod. At the end of the IAP, aphids were removed from the leaf discs and the inoculation cages containing the leaf discs were placed inside a humid box lined with wet paper towels and incubated for 2 additional days to allow virus replication (Plate X). BBTV was detected from the leaf discs using nested PCR as described below.

Leaf disc assays were used to assess the transmission of BBTV by the 12 lineages. The experimental design included six randomized incomplete blocks, testing at each time BBTV transmission by eight *Pentalonia* lineages. Each block was performed every 6 days. Transmission rates were determined by inoculating 10 leaf discs in each of 4 replicates. Controls consisted of aphids that were collected from healthy plants and that were confined to leaf discs. For each lineage tested, two leaf discs served as a negative control.

Plant transmission assay

To support the findings of the leaf disc assay, transmission experiments on 2-3 week old banana plantlets were carried out using 4 of the lineages: B_4, G_4, H_3, and T_1. These four lineages represented the most prevalent genotypes and one taro lineage. Lineages tested originated from distinct host plants. Fourth instar aphids were collected from rearing colonies and allowed to feed on BBTV infected leaf cuttings for a 4 day

AAP, as previously described (Plate IX). At the end of the AAP, groups of 3 aphids from each colony were transferred to healthy banana plants at growing stage of 3-5 leaves for an IAP of 2 days Plate XI. Ten plants were assayed per treatment. The experiment was replicated 4 times. At the end of the IAP, aphids were removed from the plants. Plants were then sprayed with .0120 % imidacloprid (Bayer Rose and Flower Insect Killer, Research Triangle Park, NC) and were transferred to insect-proof cages (Bioquip; 35.5 x 35.5 x 70 cm) in a greenhouse. Plants were observed for 85 days and then visually inspected for BBTV symptom expression. Plants not displaying typical bunchy top symptoms were considered to have escaped transmission.

BBTV detection and statistical analysis

DNA was extracted from leaf discs using a mortar and pestle and Cetyltrimethylammonium bromide (CTAB) buffer following general extraction procedures for DNA extraction from plant tissues (Xie and Hu, 1995). The presence of BBTV was confirmed using a nested PCR assay. A first round of amplification was performed using 1 µl of DNA in 25 µl of master mix, containing 5 pmol of primers 73F (5′-GGCTTTATCCAGAAGACCAA-3′) and 73R (5′-CCGTATCATGTATATTTGTTT-3′) to specifically detect BBTV genomic component S. The PCR products obtained from the first round of amplification were diluted 30 times and 1 µl of the diluted product was used in a second round of amplification, with a master mix containing 5 pmol of each primer 1004 (5’-GCTAGGTATCCGAAGAAATCC-3’) and 1005 (5’-ATAAAGCTTTCAAAC ATGATATGT-3’) to amplify an internal portion of BBTV component S produced by direct PCR. Cycling parameters for first and second round of amplification were as follows: 2’ at 94°, followed by 35 cycles of 1’ at 94°, 1’ at 50°, 1’ at 72°, then 2 holds at 72° for 5’ and 4°∞. All PCR products were separated through gel electrophoresis in a 1% agarose gel following standard protocols. For the plant assay, plants not showing visual symptoms of BBTV infection after 85 days post inoculation were considered to have escaped transmission. Leaf disc transmission data were arcsine transformed to allow for analysis of variance using GLM in SAS. The fixed effects were original host plant, genotype and species. Block was considered a random effect. When effects were significant, a Tukey’s test was used to compare means (*P* <.05).

Results

Aphid identification

Table 1 (in Chapter 2) reports the identification of species and genotype for the 12 laboratory established asexual lineages. Sequencing a portion of the COI gene followed by a blast search in GenBank identified *P. nigronervosa* collected from banana plants and *P. caladii* collected from alternative host plants (Chapter 2; Table 1). Based on 9 microsatellite markers, 7 distinct MLGs were assigned using the software Genotype and Genodive. Two MLG's, P_1 and P_3, were collected from banana and belonged to *P. nigronervosa*. Five MLG's, P_2, P_4, P_5, P_6, and P_7, were collected from red ginger, heliconia and taro species and belonged to *P. nigronervosa* (Chapter 2; Table 1).

Leaf disc transmission assay

All lineages tested were able to transmit BBTV to banana leaf discs (Table 6.) GLM in SAS showed no significant differences in transmission efficiency of BBTV between *P. nigronervosa* and *P. caladii* ($F_{3, 38}$ =.57, P = .456, and among MLG's ($F_{6, 35}$=1.69, P = .154; Table 6**.**) Original aphid host plant did affect transmission, with aphids originating from taro displaying significantly higher transmission rates than aphids originating from heliconia ($F_{3, 9}$=3.24, P = .033).

Plant transmission assay

Only the *P. nigronervosa* lineage B_4 and the *P. caladii* lineage T_1 were able to transmit BBTV to banana plantlets (Table 7) although, transmission rates were not significantly different ($F_{3, 9}$ = 2.72, P = .107). *Pentalonia caladii* from red ginger and heliconia failed to transmit BBTV in any of the 4 replicates. Symptoms of BBTV started to appear in infected plants as early as 3 weeks post inoculation. First symptoms that appeared were hooking at the base of the newest unfurled leaf on either side of the midrib (Plate XII), followed by dark green streaks on the petioles (Plate XIII). In many circumstances, dwarfing was not evident until several weeks after exhibiting the first symptoms. Plate XIV illustrates a typical healthy and BBTV infected plant one month post-inoculation resulting from the transmission assays. During the first replicate, one plant did not exhibit symptoms until after 85 days.

Discussion

Pentalonia caladii displayed similar vector competence as *P. nigronervosa* when leaf discs were used. Unlike original host plants, aphid species and genotype did not appear to significantly affect rates of BBTV transmission to leaf discs. Aphids originally from taro transmitted BBTV to banana at higher rates than aphids from heliconia. However, the results obtained for the plant transmission assay did not support those obtained in the leaf disc assay. In fact, lineages of *P. caladii* from red ginger and heliconia failed to transmit BBTV to the banana plantlets. The cause of discrepancies in transmission rates observed are unknown but may include: temperature, pre-acquisition feeding and BBTV source material. Although, it is quite possible that plantlets may have a different immune response than excised leaves causing anti-xenotic effects.

Temperature is known to affect transmission efficiency of circulative plant viruses including for instance BYDV, *Pea enation mosaic virus* (PEMV), *Beet western yellows virus* (BWYV) (Sylvester, 1980, Lowles et al., 1996) and BBTV (Wu and Su, 1990b, Anhalt and Almeida, 2008). For leaf disc and plantlet transmission assays, I used very similar experimental conditions. Aphids were allowed a 4-day AAP on BBTV infected detached leaves followed by a 2-day IAP. However, the IAP for the leaf disc and plant assay was performed under different temperature regimes. The leaf disc assays were conducted within an environmental growth chamber at 25 ± 1 °C, whereas healthy banana plants were inoculated under cooler ambient temperature conditions of the laboratory (22 ± 3°C). Anhalt and Almeida (2008) evaluated *P. nigronervosa* transmission efficiency at three constant temperatures (20, 25 and 27°C) and found an AAP/IAP of 20°C resulted in significantly lower transmission efficiency (less than 30%) than at higher temperatures (60-80%). In addition, vector transmission by *P. nigronervosa* is more dependent upon temperature during the IAP than the AAP (Anhalt and Almeida, 2008). The same authors detected a 20 hour latent period within *P. nigronervosa* (at 25°C), and hypothesized the latent period would increase at lower temperatures (Anhalt and Almeida, 2008) much like the latent period of other circulative viruses (Sylvester, 1980). The effect of temperature on *P. caladii* biology and transmission efficiency of BBTV is unknown. Yet, the different temperature regimes may not completely explain the differences in transmission rates observed, especially since one lineage of each *P. caladii* and *P. nigronervosa* succeeded (albeit at lower efficiency) in transmitting the virus under ambient conditions to plantlets.

Aphids in the leaf disc trials were allowed a 24 hour pre-acquisition feed on their respective host plants in Petri dishes within an incubator at 25°C ± 1 °C, whereas the 4 lineages in the plant assay were fed on entire greenhouse plants at 26°C ± 5 °C. Disturbing aphids on entire plants may have led to agitation and an increase in production of alarm pheromone. Alarm pheromone increases mobility of aphids (Davis and Radcliffe, 2008) which could decrease time spent feeding on phloem tissues. It may be helpful in the future to designate a single pre-acquisition host plant such as banana and to rear aphids under constant conditions within a growth chamber prior to the start of experiments. But, still the fact that one lineage each of *P. caladii* and *P. nigronervosa* succeeded in transmitting BBTV suggests pre-acquisition feeding conditions did not critically affect the transmission experiments.

Virus titre within infected source tissues is also known to affect the quantity of virions acquired by vectors (Gray et al., 1991). The actual titre of virus within BBTV source tissues was not quantified. In addition, virus presence may not have been uniform within entire leaves. According to Bencharki et al. (2000), viral particles may be packaged in aggregates allowing more efficient acquisition of *Barley yellow dwarf virus* (BYDV) within certain phloem tissues. Future studies may quantify virus titre using real-time PCR prior to using infected tissues in experiments. It would also be helpful test the virus titre in aphids. These assays may shed light on some of the differences in transmission that occurred between aphids from alternative host plants for the two transmission assays.

For transmission of BBTV to plantlets, the last 2 replicates resulted in extremely low transmissions (0 or 2 plants infected). The source of BBTV infected banana was used 2 months post inoculation in leaf disc assays and the first two replicates of plant assays. However, BBTV source plants used in the 3rd and 4th replicate were considerably younger and had been sprayed with imidacloprid 2.5 months prior to exposure to aphids. Imidacloprid may have led to anti-feedant effects. In other studies, when plants were grown with a seed application of imidacloprid aphids were less likely to transmit *Potato leaf roll virus* (PLRV) (Woodford, 1992, Dewar et al., 1992).

Differences in feeding behavior among clonal lineages may explain differences in the transmission observed. In previous colonization trials (Chapter 2) *P. caladii* from red ginger and heliconia were able to colonize banana plants over a 30 day period. However, this does not provide information on the feeding behavior of *P. caladii* on banana. For example, some aphids require a longer period of time to reach phloem tissues or may spend a larger amount of time ingesting plant sap than releasing saliva through which virions are inoculated to the plant tissues (Sylvester, 1980). The use of the electronic penetration graph (EPG) may help explaining the inability of *P. caladii* aphids from red ginger and heliconia to transmit BBTV in plantlet assays. Other authors have used EPG to determine transmission efficiency of plant viruses by aphids (Boquel et al., 2011) and found intra-specific differences in reaching the phloem and in the amount of sap ingested. Persistent circulative viruses may be released within non vascular tissues (Sylvester, 1980). Thus it may be possible that *P. caladii* from ginger and heliconia transmitted BBTV to non-vascular tissues, but failed to inoculate the virus within phloem cells that are suitable infection cells for BBTV. This may explain the local detection of virus on the leaf discs but the failure to establish an infection on the entire plant. We used a nested PCR to detect BBTV, and although we included controls to ensure detection of any contamination, the magnitude of amplification may lead to false positives, which is another point to consider when using leaf disc assays.

The number of aphids used during inoculation and age of plants can affect transmission rates (Wu and Su, 1990b). When one *P. nigronervosa* aphid was used per one month old plant in combination with a 2 day AAP/IAP, aphids were able to transmit at about 53%; but when two aphids were used to inoculate four month old plants under the same transmission conditions, only 8% of plants became BBTV infected (Wu and Su, 1990b). Within both of my transmission assays, approximately forty aphids were allowed to acquire BBTV from infected excised banana leaves within small Petri dishes (55 x 14 mm). Aphids did not appear to be crowded, however it is possible there may have been some competition that may have contributed to the low observed transmission rates. The rate of transmission is a reflection of the ability of groups of three aphids to inoculate the virus; therefore the actual BBTV transmission rates per individual aphid may be different. It seems reasonable that an increase in the number of aphid vectors would likewise increase the transmission efficiency in this study (Anhalt and Almeida, 2008, Wu and Su, 1990b). This is especially true in plant assays where aphids are not constrained to host plants and may spend a considerable amount of time walking around the cage, especially if the plant is a sub-optimal host. *Rhopalosiphum padi*, an inefficient vector of BYDV-MAV isolate was able to transmit BYDV at high rates when using 3 aphids per plant and was able to transmit multiple virus isolates when using groups of 8 aphids (Sadeghi et al., 1997). Likewise, Wu and Su (1990b) reported an increase in transmission when more than one aphid was used for inoculation of BBTV to plants of the same age. Conversely, Davis and Radcliff (2008) found that *Aphis gylcines*, the soybean aphid acquired PLRV at higher efficiency when using one aphid (78%) than when using groups of three aphids (13%) in transmission assays. Future researchers should consider the pros and cons of using groups of aphids over single aphids to in vector transmission assays.

This work is intended to inform banana farmers and extension workers where banana is an economically important staple crop about potential vectors of BBTV on alternative host plants in proximity to banana plantations. BBTV is a major threat to the vitality of Hawaii’s banana farms. Although *P. caladii* aphids from ginger and heliconia were not able to transmit BBTV to banana plantlets, the *P. caladii* lineage from taro displayed similar transmission rates as *P. nigronervosa* in plantlet trials. In the previous chapter, I demonstrated that this same *P. caladii* lineage from taro colonized potted banana, but did not create populations as large as aphids from other host plants. This taro lineage was greenish in appearance and therefore is probably a biotype. Additional BBTV-transmission assays to banana plantlets using *P. caladii* from alternative host plants are needed. Although, *Pentalonia caladii*. secondarily colonizes banana, these aphids may have great epidemiological importance in the spread of BBTV, because potential vectors can migrate to banana plants when plant quality decreases or crowding occurs.

Tables

Table 6. Mean ± SE (%) of BBTV positive banana leaf discs assessed by Nested PCR according to species (*P. nigronervosa, P. caladii*), MLG (P1, P2, P3, P4, P5, P6, P7) and original host plant (ginger, heliconia, taro, banana).

Species	Host Plant	MLG's	Total BBTV +*	Mean Transmission (%)**
P. nigronervosa	banana	P1, P3	52/120	43 ± 10
P. caladii	ginger, heliconia, taro	P2, P4, P5, P6, P7	182/344	53 ± 5
P. nigronervosa	banana	P1	18/40	45 ± 19
P. caladii	heliconia	P2	31/79	39 ± 11
P. nigronervosa	banana	P3	34/80	43 ± 12
P. caladii	Ginger	P4	68/148	46 ± 7
P. caladii	taro	P5	33/40	83 ± 5
P. caladii	taro	P6	27/38	73 ± 16
P. caladii	taro	P7	23/39	60 ± 21
P. nigronervosa	banana	P1, P3	52/120	43 ± 10ab
P. caladii	ginger	P4	55/118	46 ± 6ab
P. caladii	heliconia	P2, P4	44/109	40 ± 10b
P. caladii	taro	P5, P6, P7	83/117	72 ± 9a

*Number of leaf discs testing positive for BBTV (numerator) out of the total number of leaf discs tested (denominator).

**Means were calculated from 4 replicates. Means with different letters are significantly different at P< .05 according to Tukey's Pairwise comparisons test. Species identifications were based on Mitochondrial COI sequences. MLG's were designated by 9 microsatellite markers followed by an assessment using Genotype and Genodive software .

Table 7. Mean ± SE (%) of BBTV positive banana plantlets infected by Pentalonia aphids originally collected from banana, ginger, heliconia, and taro

Colony ID	Original Host	Rep 1	Rep 2	Rep 3	Rep 4	Transmission %
B4	Banana	1/10	5/10	0/10	0/10	15 ± 12
G4	Ginger	0/10	0/10	0/10	0/10	0
H3	Heliconia	0/10	0/10	0/10	0/10	0
T1	Taro	6/10	1/10	0/10	2/10	23 ± 13

Plates

Plate VIII. Healthy plants displaying banana hands (left) and BBTV infected banana plants (right).

Plate IX. Forty adult Pentalonia aphids were given a 4 day acquisition access period (AAP) on BBTV infected leaves within Petri dishes prior to leaf disc and plantlet transmission.

Plate X. Ten groups of 3 Pentalonia aphids were transferred to inoculation cages for a 2 day inoculation access period (IAP) on healthy banana leaf discs. After 2 days aphids were removed and leaf discs were placed back in the incubator to increase virus titre. BBTV presence was detected by Nested PCR.

Plates (Continued)

Plate XI. Ten groups of 3 *Pentalonia* aphids were transferred to 3-4 week old healthy banana plantlets for a 2 day inoculation access period (IAP). After 2 days aphids were removed and plants were moved to a greenhouse. BBTV presence was confirmed by visual inspection of symptoms after 85 days.

Plate XII. Initial symptoms of BBTV infection (hooking on midrib) during whole plant transmission assays (left) and healthy banana leaf (right).

Plate XIII. Initial symptoms of BBTV infection (streaking of petiole) during whole plant transmission assays (left) and healthy banana petiole (right).

Plates (Continued)

Plate XIV. Healthy banana plant (left) and BBTV infected banana plant (right) one month post inoculation of BBTV by groups of 3 adult aphids.

Appendices

Appendix 1. Definitions of life history and developmental parameters evaluated for *P. nigronervosa and P. caladii* reared on banana leaf discs at 25°C.

Parameter Tested	***Definition***
Intrinsic Rate of Increase (r_m)	# births- #deaths/GT
Net Reproductive Rate (ro)	# births/# adult females/GT
Doubling Time (DT)	period for offspring to double in size
Generation Time (T)	Avg. time to increase by a factor equal to Ro
Mean Development Time	Avg. time to reach adulthood
Lifespan	Avg. total days lived
Fecundity	Avg. offspring/female/day
Total Offspring	Avg. total offspring in lifetime
Adult Longevity	total days lived past nymphal period
Nymphal Mortality	% deaths before reaching adulthood

Appendix 2. Polymorphic loci used to differentiate MLG's of *P. nigronervosa* and *P. caladii.*

Clone ID	Genotype	M62		S24		Ago66		S17b		S16b		S23		AF169		AF-4		AF-I	
B1	1	101	117	161	161	97	141	139	139	207	207	107	111	159	159	81	89	0	0
B3	3	105	111	155	155	129	129	139	139	0	0	107	111	155	155	75	111	271	271
B4	3	105	111	155	155	101	101	139	139	0	0	107	111	155	155	75	111	271	271
G1	4	95	99	149	149	101	129	123	129	207	207	107	111	155	155	75	75	271	271
G3	4	95	99	149	149	101	129	123	129	207	207	107	111	155	155	75	111	271	271
G4	4	95	99	149	149	101	129	123	129	207	207	107	111	155	155	75	111	271	271
H1	4	105	111	161	161	101	139	133	139	207	207	107	111	155	155	75	111	271	271
H2	2	95	99	149	149	101	129	123	129	207	207	107	111	155	155	75	111	271	271
H3	2	105	111	161	161	101	139	133	139	207	207	107	111	155	155	75	111	271	271
T1	5	117	121	143	161	97	139	133	139	207	207	107	111	155	155	75	111	271	271
T2	6	117	121	143	161	97	139	133	139	207	207	117	117	159	159	97	97	0	0
T3	7	111	117	151	161	97	139	139	139	207	207	117	119	159	159	81	97	0	0

Appendix 3. Complete life table for *P. caladii* on banana leaf discs at 25°C.

Age Class, x	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
0	1.000	1.000	0.000	0.000	1.000	33.737	33.737	304
1	1.000	1.000	0.000	0.000	1.000	32.737	32.737	304
2	1.000	0.990	0.010	0.010	0.995	31.737	31.737	304
3	0.990	0.983	0.017	0.016	0.982	30.742	31.048	301
4	0.974	0.990	0.010	0.010	0.969	29.760	30.564	296
5	0.964	0.993	0.007	0.007	0.961	28.791	29.872	293
6	0.957	0.993	0.007	0.007	0.954	27.831	29.074	291
7	0.951	0.990	0.010	0.010	0.946	26.877	28.272	289
8	0.941	0.997	0.003	0.003	0.939	25.931	27.563	286
9	0.938	0.996	0.004	0.003	0.936	24.992	26.658	285
10	0.934	1.000	0.000	0.000	0.934	24.056	25.750	284
11	0.934	0.996	0.004	0.003	0.933	23.122	24.750	284
12	0.931	0.996	0.004	0.003	0.929	22.189	23.836	283
13	0.928	0.996	0.004	0.003	0.926	21.260	22.918	282
14	0.924	0.996	0.004	0.003	0.923	20.334	21.998	281
15	0.921	0.996	0.004	0.003	0.919	19.411	21.075	280
16	0.918	1.000	0.000	0.000	0.918	18.492	20.149	279
17	0.918	1.000	0.000	0.000	0.918	17.574	19.149	279
18	0.918	1.000	0.000	0.000	0.918	16.656	18.149	279
19	0.918	0.989	0.011	0.010	0.913	15.738	17.149	279
20	0.908	0.993	0.007	0.007	0.905	14.826	16.330	276
21	0.901	0.993	0.007	0.007	0.898	13.921	15.445	274
22	0.895	0.989	0.011	0.010	0.890	13.023	14.555	272
23	0.885	0.978	0.022	0.020	0.875	12.133	13.712	269
24	0.865	0.970	0.030	0.026	0.852	11.258	13.013	263
25	0.839	0.980	0.020	0.016	0.831	10.406	12.406	255
26	0.822	0.976	0.024	0.020	0.813	9.576	11.644	250
27	0.803	0.992	0.008	0.007	0.799	8.763	10.918	244
28	0.796	0.955	0.045	0.036	0.778	7.964	10.004	242
29	0.760	0.961	0.039	0.030	0.745	7.186	9.457	231
30	0.730	0.959	0.041	0.030	0.715	6.441	8.820	222
31	0.701	0.948	0.052	0.036	0.683	5.725	8.171	213
32	0.664	0.955	0.045	0.030	0.650	5.043	7.589	202
33	0.635	0.938	0.062	0.039	0.615	4.393	6.920	193
34	0.595	0.906	0.094	0.056	0.567	3.778	6.345	181
35	0.539	0.939	0.061	0.033	0.523	3.211	5.951	164
36	0.507	0.909	0.091	0.046	0.484	2.688	5.305	154
37	0.461	0.907	0.093	0.043	0.439	2.204	4.786	140

Appendix 3. (Continued) Complete Life Table for *P. caladii* on banana leaf discs at 25°C.

Age Class, x	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
38	0.418	0.827	0.173	0.072	0.382	1.765	4.224	127
39	0.345	0.771	0.229	0.079	0.306	1.383	4.005	105
40	0.266	0.926	0.074	0.020	0.257	1.077	4.043	81
41	0.247	0.787	0.213	0.053	0.220	0.821	3.327	75
42	0.194	0.814	0.186	0.036	0.176	0.600	3.093	59
43	0.158	0.813	0.188	0.030	0.143	0.424	2.688	48
44	0.128	0.641	0.359	0.046	0.105	0.281	2.192	39
45	0.082	0.680	0.320	0.026	0.069	0.176	2.140	25
46	0.056	0.588	0.412	0.023	0.044	0.107	1.912	17
47	0.033	0.800	0.200	0.007	0.030	0.063	1.900	10
48	0.026	0.500	0.500	0.013	0.020	0.033	1.250	8
49	0.013	0.500	0.500	0.007	0.010	0.013	1.000	4
50	0.007	0.000	1.000	0.007	0.003	0.003	0.500	2
51	0.000			0.000	0.000	0.000		0

Appendix 4. Complete life table for P. nigronervosa on banana leaf discs at 25°C.

Age Class, x	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
0	1.000	1.000	0.000	0.000	1.000	30.780	30.780	100
1	1.000	0.990	0.010	0.010	0.995	29.780	29.780	100
2	0.990	1.000	0.000	0.000	0.990	28.785	29.076	99
3	0.990	0.990	0.010	0.010	0.985	27.795	28.076	99
4	0.980	0.969	0.031	0.030	0.965	26.810	27.357	98
5	0.950	1.000	0.000	0.000	0.950	25.845	27.205	95
6	0.950	1.000	0.000	0.000	0.950	24.895	26.205	95
7	0.950	1.000	0.000	0.000	0.950	23.945	25.205	95
8	0.950	1.000	0.000	0.000	0.950	22.995	24.205	95
9	0.950	0.979	0.021	0.020	0.940	22.045	23.205	95
10	0.930	1.000	0.000	0.000	0.930	21.105	22.694	93
11	0.930	0.989	0.011	0.010	0.925	20.175	21.694	93
12	0.920	1.000	0.000	0.000	0.920	19.250	20.924	92
13	0.920	0.989	0.011	0.010	0.915	18.330	19.924	92
14	0.910	1.000	0.000	0.000	0.910	17.415	19.137	91
15	0.910	1.000	0.000	0.000	0.910	16.505	18.137	91
16	0.910	1.000	0.000	0.000	0.910	15.595	17.137	91
17	0.910	1.000	0.000	0.000	0.910	14.685	16.137	91
18	0.910	1.000	0.000	0.000	0.910	13.775	15.137	91
19	0.910	0.989	0.011	0.010	0.905	12.865	14.137	91
20	0.900	0.989	0.011	0.010	0.895	11.960	13.289	90
21	0.890	1.000	0.000	0.000	0.890	11.065	12.433	89
22	0.890	0.978	0.022	0.020	0.880	10.175	11.433	89
23	0.870	0.977	0.023	0.020	0.860	9.295	10.684	87
24	0.850	0.965	0.035	0.030	0.835	8.435	9.924	85
25	0.820	0.927	0.073	0.060	0.790	7.600	9.268	82
26	0.760	0.974	0.026	0.020	0.750	6.810	8.961	76
27	0.740	0.892	0.108	0.080	0.700	6.060	8.189	74
28	0.660	0.939	0.061	0.040	0.640	5.360	8.121	66
29	0.620	0.984	0.016	0.010	0.615	4.720	7.613	62
30	0.610	0.918	0.082	0.050	0.585	4.105	6.730	61
31	0.560	0.875	0.125	0.070	0.525	3.520	6.286	56
32	0.490	0.939	0.061	0.030	0.475	2.995	6.112	49
33	0.460	0.870	0.130	0.060	0.430	2.520	5.478	46
34	0.400	0.950	0.050	0.020	0.390	2.090	5.225	40
35	0.380	0.868	0.132	0.050	0.355	1.700	4.474	38
36	0.330	0.818	0.182	0.060	0.300	1.345	4.076	33
37	0.270	0.778	0.222	0.060	0.240	1.045	3.870	27

Appendix 4. (Continued) Complete life table for *P. nigronervosa* on banana leaf discs at 25°C.

Age Class, x	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
38	0.210	0.714	0.286	0.060	0.180	0.805	3.833	21
39	0.150	0.867	0.133	0.020	0.140	0.625	4.167	15
40	0.130	0.923	0.077	0.010	0.125	0.485	3.731	13
41	0.120	0.917	0.083	0.010	0.115	0.360	3.000	12
42	0.110	0.909	0.091	0.010	0.105	0.245	2.227	11
43	0.100	0.400	0.600	0.060	0.070	0.140	1.400	10
44	0.040	0.250	0.750	0.030	0.025	0.070	1.750	4
45	0.010	1.000	0.000	0.000	0.010	0.045	4.500	1
46	0.010	1.000	0.000	0.000	0.010	0.035	3.500	1
47	0.010	1.000	0.000	0.000	0.010	0.025	2.500	1
48	0.010	1.000	0.000	0.000	0.010	0.015	1.500	1
49	0.010	0.000	1.000	0.010	0.005	0.005	0.500	1
50	0.000			0.000	0.000	0.000		

Appendix 5. Complete life table for P1 reared on banana leaf discs at 25°C.

Age Class, x	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
0	1.000	1.000	0.000	0.000	1.000	30.643	30.643	35
1	1.000	1.000	0.000	0.000	1.000	29.643	29.643	35
2	1.000	1.000	0.000	0.000	1.000	28.643	28.643	35
3	1.000	1.000	0.000	0.000	1.000	27.643	27.643	35
4	1.000	1.000	0.000	0.000	1.000	26.643	26.643	35
5	1.000	1.000	0.000	0.000	1.000	25.643	25.643	35
6	1.000	1.000	0.000	0.000	1.000	24.643	24.643	35
7	1.000	1.000	0.000	0.000	1.000	23.643	23.643	35
8	1.000	1.000	0.000	0.000	1.000	22.643	22.643	35
9	1.000	0.943	0.057	0.057	0.971	21.643	21.643	35
10	0.943	1.000	0.000	0.000	0.943	20.671	21.924	33
11	0.943	0.970	0.030	0.029	0.929	19.729	20.924	33
12	0.914	1.000	0.000	0.000	0.914	18.800	20.563	32
13	0.914	0.969	0.031	0.029	0.900	17.886	19.563	32
14	0.886	1.000	0.000	0.000	0.886	16.986	19.177	31
15	0.886	1.000	0.000	0.000	0.886	16.100	18.177	31
16	0.886	1.000	0.000	0.000	0.886	15.214	17.177	31
17	0.886	1.000	0.000	0.000	0.886	14.329	16.177	31
18	0.886	1.000	0.000	0.000	0.886	13.443	15.177	31
19	0.886	1.000	0.000	0.000	0.886	12.557	14.177	31
20	0.886	0.968	0.032	0.029	0.871	11.671	13.177	31
21	0.857	1.000	0.000	0.000	0.857	10.800	12.600	30
22	0.857	0.967	0.033	0.029	0.843	9.943	11.600	30
23	0.829	1.000	0.000	0.000	0.829	9.100	10.983	29
24	0.829	0.966	0.034	0.029	0.814	8.271	9.983	29
25	0.800	0.929	0.071	0.057	0.771	7.457	9.321	28
26	0.743	0.962	0.038	0.029	0.729	6.686	9.000	26
27	0.714	0.960	0.040	0.029	0.700	5.957	8.340	25
28	0.686	1.000	0.000	0.000	0.686	5.257	7.667	24
29	0.686	1.000	0.000	0.000	0.686	4.571	6.667	24
30	0.686	0.917	0.083	0.057	0.657	3.886	5.667	24
31	0.629	0.818	0.182	0.114	0.571	3.229	5.136	22
32	0.514	0.944	0.056	0.029	0.500	2.657	5.167	18
33	0.486	0.824	0.176	0.086	0.443	2.157	4.441	17
34	0.400	1.000	0.000	0.000	0.400	1.714	4.286	14
35	0.400	0.786	0.214	0.086	0.357	1.314	3.286	14
36	0.314	0.727	0.273	0.086	0.271	0.957	3.045	11

Appendix 5. (Continued) Complete life table for P1 reared on banana leaf discs at 25°C.

Age Class, x	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
37	0.229	0.625	0.375	0.086	0.186	0.686	3.000	8
38	0.143	0.600	0.400	0.057	0.114	0.500	3.500	5
39	0.086	1.000	0.000	0.000	0.086	0.386	4.500	3
40	0.086	1.000	0.000	0.000	0.086	0.300	3.500	3
41	0.086	0.667	0.333	0.029	0.071	0.214	2.500	3
42	0.057	1.000	0.000	0.000	0.057	0.143	2.500	2
43	0.057	1.000	0.000	0.000	0.057	0.086	1.500	2
44	0.057	0.000	1.000	0.057	0.029	0.029	0.500	2
45	0.000			0.000	0.000			0

Appendix 6. Complete life table for P2 reared on banana leaf discs at 25°C.

Age Class, x	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
0	1.000	1.000	0.000	0.000	1.000	36.688	36.688	48
1	1.000	1.000	0.000	0.000	1.000	35.688	35.688	48
2	1.000	1.000	0.000	0.000	1.000	34.688	34.688	48
3	1.000	1.000	0.000	0.000	1.000	33.688	33.688	48
4	1.000	1.000	0.000	0.000	1.000	32.688	32.688	48
5	1.000	1.000	0.000	0.000	1.000	31.688	31.688	48
6	1.000	1.000	0.000	0.000	1.000	30.688	30.688	48
7	1.000	1.000	0.000	0.000	1.000	29.688	29.688	48
8	1.000	1.000	0.000	0.000	1.000	28.688	28.688	48
9	1.000	1.000	0.000	0.000	1.000	27.688	27.688	48
10	1.000	1.000	0.000	0.000	1.000	26.688	26.688	48
11	1.000	1.000	0.000	0.000	1.000	25.688	25.688	48
12	1.000	1.000	0.000	0.000	1.000	24.688	24.688	48
13	1.000	1.000	0.000	0.000	1.000	23.688	23.688	48
14	1.000	1.000	0.000	0.000	1.000	22.688	22.688	48
15	1.000	1.000	0.000	0.000	1.000	21.688	21.688	48
16	1.000	1.000	0.000	0.000	1.000	20.688	20.688	48
17	1.000	1.000	0.000	0.000	1.000	19.688	19.688	48
18	1.000	1.000	0.000	0.000	1.000	18.688	18.688	48
19	1.000	1.000	0.000	0.000	1.000	17.688	17.688	48
20	1.000	1.000	0.000	0.000	1.000	16.688	16.688	48
21	1.000	1.000	0.000	0.000	1.000	15.688	15.688	48
22	1.000	1.000	0.000	0.000	1.000	14.688	14.688	48
23	1.000	0.979	0.021	0.021	0.990	13.688	13.688	48
24	0.979	0.936	0.064	0.063	0.948	12.698	12.968	47
25	0.917	1.000	0.000	0.000	0.917	11.750	12.818	44
26	0.917	1.000	0.000	0.000	0.917	10.833	11.818	44
27	0.917	1.000	0.000	0.000	0.917	9.917	10.818	44
28	0.917	0.977	0.023	0.021	0.906	9.000	9.818	44
29	0.896	0.930	0.070	0.063	0.865	8.094	9.035	43
30	0.833	0.950	0.050	0.042	0.813	7.229	8.675	40
31	0.792	0.974	0.026	0.021	0.781	6.417	8.105	38
32	0.771	0.946	0.054	0.042	0.750	5.635	7.311	37
33	0.729	0.971	0.029	0.021	0.719	4.885	6.700	35
34	0.708	0.941	0.059	0.042	0.688	4.167	5.882	34
35	0.667	0.906	0.094	0.063	0.635	3.479	5.219	32

Appendix 6. (Continued) Complete life table for P2 reared on banana leaf discs at 25°C.

Age Class, x	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
36	0.604	0.966	0.034	0.021	0.594	2.844	4.707	29
37	0.583	0.857	0.143	0.083	0.542	2.250	3.857	28
38	0.500	0.792	0.208	0.104	0.448	1.708	3.417	24
39	0.396	0.684	0.316	0.125	0.333	1.260	3.184	19
40	0.271	0.846	0.154	0.042	0.250	0.927	3.423	13
41	0.229	0.727	0.273	0.063	0.198	0.677	2.955	11
42	0.167	0.875	0.125	0.021	0.156	0.479	2.875	8
43	0.146	0.857	0.143	0.021	0.135	0.323	2.214	7
44	0.125	0.667	0.333	0.042	0.104	0.188	1.500	6
45	0.083	0.500	0.500	0.042	0.063	0.083	1.000	4
46	0.042	0.000	1.000	0.042	0.021	0.021	0.500	2
47	0.000			0.000	0.000	0.000		0

Appendix 7. Complete life table for P3 reared on banana leaf discs at 25°C.

Age Class, x	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
0	1.000	1.000	0.000	0.000	1.000	30.854	30.854	65
1	1.000	0.985	0.015	0.015	0.992	29.854	29.854	65
2	0.985	1.000	0.000	0.000	0.985	28.862	29.313	64
3	0.985	0.984	0.016	0.015	0.977	27.877	28.313	64
4	0.969	0.952	0.048	0.046	0.946	26.900	27.754	63
5	0.923	1.000	0.000	0.000	0.923	25.954	28.117	60
6	0.923	1.000	0.000	0.000	0.923	25.031	27.117	60
7	0.923	1.000	0.000	0.000	0.923	24.108	26.117	60
8	0.923	1.000	0.000	0.000	0.923	23.185	25.117	60
9	0.923	1.000	0.000	0.000	0.923	22.262	24.117	60
10	0.923	1.000	0.000	0.000	0.923	21.338	23.117	60
11	0.923	1.000	0.000	0.000	0.923	20.415	22.117	60
12	0.923	1.000	0.000	0.000	0.923	19.492	21.117	60
13	0.923	1.000	0.000	0.000	0.923	18.569	20.117	60
14	0.923	1.000	0.000	0.000	0.923	17.646	19.117	60
15	0.923	1.000	0.000	0.000	0.923	16.723	18.117	60
16	0.923	1.000	0.000	0.000	0.923	15.800	17.117	60
17	0.923	1.000	0.000	0.000	0.923	14.877	16.117	60
18	0.923	1.000	0.000	0.000	0.923	13.954	15.117	60
19	0.923	0.983	0.017	0.015	0.915	13.031	14.117	60
20	0.908	1.000	0.000	0.000	0.908	12.115	13.347	59
21	0.908	1.000	0.000	0.000	0.908	11.208	12.347	59
22	0.908	0.983	0.017	0.015	0.900	10.300	11.347	59
23	0.892	0.966	0.034	0.031	0.877	9.400	10.534	58
24	0.862	0.964	0.036	0.031	0.846	8.523	9.893	56
25	0.831	0.926	0.074	0.062	0.800	7.677	9.241	54
26	0.769	0.980	0.020	0.015	0.762	6.877	8.940	50
27	0.754	0.857	0.143	0.108	0.700	6.115	8.112	49
28	0.646	0.905	0.095	0.062	0.615	5.415	8.381	42
29	0.585	0.974	0.026	0.015	0.577	4.800	8.211	38
30	0.569	0.919	0.081	0.046	0.546	4.223	7.419	37
31	0.523	0.912	0.088	0.046	0.500	3.677	7.029	34
32	0.477	0.935	0.065	0.031	0.462	3.177	6.661	31
33	0.446	0.897	0.103	0.046	0.423	2.715	6.086	29
34	0.400	0.923	0.077	0.031	0.385	2.292	5.731	26

Appendix 7. (Continued) Complete life table for P3 reared on banana leaf discs at 25°C.

Age Class, x	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
35	0.369	0.917	0.083	0.031	0.354	1.908	5.167	24
36	0.338	0.864	0.136	0.046	0.315	1.554	4.591	22
37	0.292	0.842	0.158	0.046	0.269	1.238	4.237	19
38	0.246	0.750	0.250	0.062	0.215	0.969	3.938	16
39	0.185	0.833	0.167	0.031	0.169	0.754	4.083	12
40	0.154	0.900	0.100	0.015	0.146	0.585	3.800	10
41	0.138	1.000	0.000	0.000	0.138	0.438	3.167	9
42	0.138	0.889	0.111	0.015	0.131	0.300	2.167	9
43	0.123	0.250	0.750	0.092	0.077	0.169	1.375	8
44	0.031	0.500	0.500	0.015	0.023	0.092	3.000	2
45	0.015	1.000	0.000	0.000	0.015	0.069	4.500	1
46	0.015	1.000	0.000	0.000	0.015	0.054	3.500	1
47	0.015	1.000	0.000	0.000	0.015	0.038	2.500	1
48	0.015	1.000	0.000	0.000	0.015	0.023	1.500	1
49	0.015	0.000	1.000	0.015	0.008	0.008	0.500	1
50	0.000			0.000	0.000	0.000		0

Appendix 8. Complete life table for P4 reared on banana leaf discs at 25°C.

Age Class, x	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
0	1.000	1.000	0.000	0.000	1.000	36.622	36.622	148
1	1.000	1.000	0.000	0.000	1.000	35.622	35.622	148
2	1.000	1.000	0.000	0.000	1.000	34.622	34.622	148
3	1.000	1.000	0.000	0.000	1.000	33.622	33.622	148
4	1.000	1.000	0.000	0.000	1.000	32.622	32.622	148
5	1.000	0.993	0.007	0.007	0.997	31.622	31.622	148
6	0.993	0.993	0.007	0.007	0.990	30.625	30.833	147
7	0.986	1.000	0.000	0.000	0.986	29.635	30.041	146
8	0.986	1.000	0.000	0.000	0.986	28.649	29.041	146
9	0.986	1.000	0.000	0.000	0.986	27.662	28.041	146
10	0.986	1.000	0.000	0.000	0.986	26.676	27.041	146
11	0.986	0.993	0.007	0.007	0.983	25.689	26.041	146
12	0.980	1.000	0.000	0.000	0.980	24.706	25.217	145
13	0.980	1.000	0.000	0.000	0.980	23.726	24.217	145
14	0.980	0.993	0.007	0.007	0.976	22.747	23.217	145
15	0.973	0.993	0.007	0.007	0.970	21.770	22.375	144
16	0.966	1.000	0.000	0.000	0.966	20.801	21.528	143
17	0.966	1.000	0.000	0.000	0.966	19.834	20.528	143
18	0.966	1.000	0.000	0.000	0.966	18.868	19.528	143
19	0.966	1.000	0.000	0.000	0.966	17.902	18.528	143
20	0.966	0.986	0.014	0.014	0.959	16.936	17.528	143
21	0.953	1.000	0.000	0.000	0.953	15.976	16.770	141
22	0.953	0.993	0.007	0.007	0.949	15.024	15.770	141
23	0.946	0.986	0.014	0.014	0.939	14.074	14.879	140
24	0.932	0.993	0.007	0.007	0.929	13.135	14.087	138
25	0.926	0.993	0.007	0.007	0.922	12.206	13.186	137
26	0.919	0.963	0.037	0.034	0.902	11.284	12.279	136
27	0.885	0.985	0.015	0.014	0.878	10.382	11.729	131
28	0.872	0.969	0.031	0.027	0.858	9.503	10.903	129
29	0.845	0.968	0.032	0.027	0.831	8.645	10.236	125
30	0.818	0.967	0.033	0.027	0.804	7.814	9.558	121
31	0.791	0.940	0.060	0.047	0.767	7.010	8.868	117
32	0.743	0.973	0.027	0.020	0.733	6.243	8.400	110
33	0.723	0.925	0.075	0.054	0.696	5.510	7.621	107
34	0.669	0.929	0.071	0.047	0.645	4.814	7.197	99
35	0.622	0.957	0.043	0.027	0.608	4.169	6.707	92
36	0.595	0.898	0.102	0.061	0.564	3.561	5.989	88
37	0.534	0.937	0.063	0.034	0.517	2.997	5.614	79
38	0.500	0.865	0.135	0.068	0.466	2.480	4.959	74

Appendix 8. (Continued) Complete life table for P4 reared on banana leaf discs at 25°C.

Age Class, X	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
39	0.432	0.813	0.188	0.081	0.392	2.014	4.656	64
40	0.351	0.962	0.038	0.014	0.345	1.622	4.615	52
41	0.338	0.840	0.160	0.054	0.311	1.277	3.780	50
42	0.284	0.833	0.167	0.047	0.260	0.966	3.405	42
43	0.236	0.857	0.143	0.034	0.220	0.706	2.986	35
44	0.203	0.633	0.367	0.074	0.166	0.486	2.400	30
45	0.128	0.737	0.263	0.034	0.111	0.321	2.500	19
46	0.095	0.714	0.286	0.027	0.081	0.209	2.214	14
47	0.068	0.800	0.200	0.014	0.061	0.128	1.900	10
48	0.054	0.500	0.500	0.027	0.041	0.068	1.250	8
49	0.027	0.500	0.500	0.014	0.020	0.027	1.000	4
50	0.014	0.000	1.000	0.014	0.007	0.007	0.500	2
51	0.000			0.000	0.000	0.000		0

Appendix 9. Complete life table for P5 reared on banana leaf discs at 25°C.

Age Class, x	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
0	1.000	1.000	0.000	0.000	1.000	28.414	28.414	35
1	1.000	1.000	0.000	0.000	1.000	27.414	27.414	35
2	1.000	0.943	0.057	0.057	0.971	26.414	26.414	35
3	0.943	0.909	0.091	0.086	0.900	25.443	26.985	33
4	0.857	0.967	0.033	0.029	0.843	24.543	28.633	30
5	0.829	1.000	0.000	0.000	0.829	23.700	28.603	29
6	0.829	1.000	0.000	0.000	0.829	22.871	27.603	29
7	0.829	1.000	0.000	0.000	0.829	22.043	26.603	29
8	0.829	1.000	0.000	0.000	0.829	21.214	25.603	29
9	0.829	1.000	0.000	0.000	0.829	20.386	24.603	29
10	0.829	1.000	0.000	0.000	0.829	19.557	23.603	29
11	0.829	1.000	0.000	0.000	0.829	18.729	22.603	29
12	0.829	1.000	0.000	0.000	0.829	17.900	21.603	29
13	0.829	1.000	0.000	0.000	0.829	17.071	20.603	29
14	0.829	1.000	0.000	0.000	0.829	16.243	19.603	29
15	0.829	1.000	0.000	0.000	0.829	15.414	18.603	29
16	0.829	1.000	0.000	0.000	0.829	14.586	17.603	29
17	0.829	1.000	0.000	0.000	0.829	13.757	16.603	29
18	0.829	1.000	0.000	0.000	0.829	12.929	15.603	29
19	0.829	0.931	0.069	0.057	0.800	12.100	14.603	29
20	0.771	1.000	0.000	0.000	0.771	11.300	14.648	27
21	0.771	0.963	0.037	0.029	0.757	10.529	13.648	27
22	0.743	0.923	0.077	0.057	0.714	9.771	13.154	26
23	0.686	0.958	0.042	0.029	0.671	9.057	13.208	24
24	0.657	1.000	0.000	0.000	0.657	8.386	12.761	23
25	0.657	1.000	0.000	0.000	0.657	7.729	11.761	23
26	0.657	1.000	0.000	0.000	0.657	7.071	10.761	23
27	0.657	1.000	0.000	0.000	0.657	6.414	9.761	23
28	0.657	1.000	0.000	0.000	0.657	5.757	8.761	23
29	0.657	0.957	0.043	0.029	0.643	5.100	7.761	23
30	0.629	1.000	0.000	0.000	0.629	4.457	7.091	22
31	0.629	1.000	0.000	0.000	0.629	3.829	6.091	22
32	0.629	0.909	0.091	0.057	0.600	3.200	5.091	22
33	0.571	0.900	0.100	0.057	0.543	2.600	4.550	20
34	0.514	0.833	0.167	0.086	0.471	2.057	4.000	18
35	0.429	0.800	0.200	0.086	0.386	1.586	3.700	15
36	0.343	0.917	0.083	0.029	0.329	1.200	3.500	12
37	0.314	0.818	0.182	0.057	0.286	0.871	2.773	11
38	0.257	0.556	0.444	0.114	0.200	0.586	2.278	9

Appendix 9. (Continued) Complete life table for P5 reared on banana leaf discs at 25°C.

Age Class, x	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
39	0.143	0.600	0.400	0.057	0.114	0.386	2.700	5
40	0.086	1.000	0.000	0.000	0.086	0.271	3.167	3
41	0.086	1.000	0.000	0.000	0.086	0.186	2.167	3
42	0.086	0.667	0.333	0.029	0.071	0.100	1.167	3
43	0.057	0.000	1.000	0.057	0.029	0.029	0.500	2
44	0.000			0.000	0.000	0.000		0

Appendix 10. Complete life table for P6 reared on banana leaf discs at 25°C.

Age Class, x	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
0	1.000	1.000	0.000	0.000	1.000	30.105	30.105	38
1	1.000	1.000	0.000	0.000	1.000	29.105	29.105	38
2	1.000	0.974	0.026	0.026	0.987	28.105	28.105	38
3	0.974	0.973	0.027	0.026	0.961	27.118	27.851	37
4	0.947	0.944	0.056	0.053	0.921	26.158	27.611	36
5	0.895	1.000	0.000	0.000	0.895	25.237	28.206	34
6	0.895	1.000	0.000	0.000	0.895	24.342	27.206	34
7	0.895	1.000	0.000	0.000	0.895	23.447	26.206	34
8	0.895	1.000	0.000	0.000	0.895	22.553	25.206	34
9	0.895	1.000	0.000	0.000	0.895	21.658	24.206	34
10	0.895	1.000	0.000	0.000	0.895	20.763	23.206	34
11	0.895	1.000	0.000	0.000	0.895	19.868	22.206	34
12	0.895	0.971	0.029	0.026	0.882	18.974	21.206	34
13	0.868	1.000	0.000	0.000	0.868	18.092	20.833	33
14	0.868	1.000	0.000	0.000	0.868	17.224	19.833	33
15	0.868	1.000	0.000	0.000	0.868	16.355	18.833	33
16	0.868	1.000	0.000	0.000	0.868	15.487	17.833	33
17	0.868	1.000	0.000	0.000	0.868	14.618	16.833	33
18	0.868	1.000	0.000	0.000	0.868	13.750	15.833	33
19	0.868	0.970	0.030	0.026	0.855	12.882	14.833	33
20	0.842	1.000	0.000	0.000	0.842	12.026	14.281	32
21	0.842	0.969	0.031	0.026	0.829	11.184	13.281	32
22	0.816	1.000	0.000	0.000	0.816	10.355	12.694	31
23	0.816	0.968	0.032	0.026	0.803	9.539	11.694	31
24	0.789	0.933	0.067	0.053	0.763	8.737	11.067	30
25	0.737	0.964	0.036	0.026	0.724	7.974	10.821	28
26	0.711	1.000	0.000	0.000	0.711	7.250	10.204	27
27	0.711	1.000	0.000	0.000	0.711	6.539	9.204	27
28	0.711	0.926	0.074	0.053	0.684	5.829	8.204	27
29	0.658	0.960	0.040	0.026	0.645	5.145	7.820	25
30	0.632	0.917	0.083	0.053	0.605	4.500	7.125	24
31	0.579	0.864	0.136	0.079	0.539	3.895	6.727	22
32	0.500	0.895	0.105	0.053	0.474	3.355	6.711	19
33	0.447	1.000	0.000	0.000	0.447	2.882	6.441	17
34	0.447	0.824	0.176	0.079	0.408	2.434	5.441	17
35	0.368	1.000	0.000	0.000	0.368	2.026	5.500	14
36	0.368	0.929	0.071	0.026	0.355	1.658	4.500	14
37	0.342	0.846	0.154	0.053	0.316	1.303	3.808	13

Appendix 10. (Continued) Complete life table for P6 reared on banana leaf discs at 25°C.

Age Class, X	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
38	0.289	1.000	0.000	0.000	0.289	0.987	3.409	11
39	0.289	0.818	0.182	0.053	0.263	0.697	2.409	11
40	0.237	0.778	0.222	0.053	0.211	0.434	1.833	9
41	0.184	0.429	0.571	0.105	0.132	0.224	1.214	7
42	0.079	0.333	0.667	0.053	0.053	0.092	1.167	3
43	0.026	1.000	0.000	0.000	0.026	0.039	1.500	1
44	0.026	0.000	1.000	0.026	0.013	0.013	0.500	1
45	0.000			0.000	0.000			0

Appendix 11. Complete life table for P7 reared on banana leaf discs at 25°C.

Age Class, X	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
0	1.000	1.000	0.000	0.000	1.000	26.757	26.757	35
1	1.000	1.000	0.000	0.000	1.000	25.757	25.757	35
2	1.000	1.000	0.000	0.000	1.000	24.757	24.757	35
3	1.000	0.971	0.029	0.029	0.986	23.757	23.757	35
4	0.971	1.000	0.000	0.000	0.971	22.771	23.441	34
5	0.971	0.971	0.029	0.029	0.957	21.800	22.441	34
6	0.943	0.970	0.030	0.029	0.929	20.843	22.106	33
7	0.914	0.906	0.094	0.086	0.871	19.914	21.781	32
8	0.829	0.966	0.034	0.029	0.814	19.043	22.983	29
9	0.800	0.964	0.036	0.029	0.786	18.229	22.786	28
10	0.771	1.000	0.000	0.000	0.771	17.443	22.611	27
11	0.771	1.000	0.000	0.000	0.771	16.671	21.611	27
12	0.771	1.000	0.000	0.000	0.771	15.900	20.611	27
13	0.771	0.963	0.037	0.029	0.757	15.129	19.611	27
14	0.743	1.000	0.000	0.000	0.743	14.371	19.346	26
15	0.743	1.000	0.000	0.000	0.743	13.629	18.346	26
16	0.743	1.000	0.000	0.000	0.743	12.886	17.346	26
17	0.743	1.000	0.000	0.000	0.743	12.143	16.346	26
18	0.743	1.000	0.000	0.000	0.743	11.400	15.346	26
19	0.743	1.000	0.000	0.000	0.743	10.657	14.346	26
20	0.743	1.000	0.000	0.000	0.743	9.914	13.346	26
21	0.743	1.000	0.000	0.000	0.743	9.171	12.346	26
22	0.743	1.000	0.000	0.000	0.743	8.429	11.346	26
23	0.743	0.962	0.038	0.029	0.729	7.686	10.346	26
24	0.714	0.920	0.080	0.057	0.686	6.957	9.740	25
25	0.657	0.870	0.130	0.086	0.614	6.271	9.543	23
26	0.571	0.950	0.050	0.029	0.557	5.657	9.900	20
27	0.543	1.000	0.000	0.000	0.543	5.100	9.395	19
28	0.543	0.789	0.211	0.114	0.486	4.557	8.395	19
29	0.429	1.000	0.000	0.000	0.429	4.071	9.500	15
30	0.429	0.933	0.067	0.029	0.414	3.643	8.500	15
31	0.400	1.000	0.000	0.000	0.400	3.229	8.071	14
32	0.400	1.000	0.000	0.000	0.400	2.829	7.071	14
33	0.400	0.929	0.071	0.029	0.386	2.429	6.071	14
34	0.371	0.846	0.154	0.057	0.343	2.043	5.500	13
35	0.314	1.000	0.000	0.000	0.314	1.700	5.409	11
36	0.314	0.818	0.182	0.057	0.286	1.386	4.409	11
37	0.257	1.000	0.000	0.000	0.257	1.100	4.278	9

Appendix 11. (Continued) Complete Life Table for P_7 reared on banana leaf discs at 25°C.

Age Class, x	Fraction Living at Age x, l_x	Fraction Surviving from x to x+1, p_x	Fraction Dying from x to x+1, q_x	Fraction Dying in Interval x to x+1, D_x	Days Lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x	Number of Adults Surviving from x to x+1, n_x
38	0.257	0.667	0.333	0.086	0.214	0.843	3.278	9
39	0.171	0.667	0.333	0.057	0.143	0.629	3.667	6
40	0.114	1.000	0.000	0.000	0.114	0.486	4.250	4
41	0.114	0.750	0.250	0.029	0.100	0.371	3.250	4
42	0.086	1.000	0.000	0.000	0.086	0.271	3.167	3
43	0.086	0.667	0.333	0.029	0.071	0.186	2.167	3
44	0.057	1.000	0.000	0.000	0.057	0.114	2.000	2
45	0.057	0.500	0.500	0.029	0.043	0.057	1.000	2
46	0.029	0.000	1.000	0.029	0.014	0.014	0.500	1
47	0.000			0.000	0.000			0

References

[NASS] National Agricultural Statistics Service. 2009. Hawaii bananas annual summary. (http://www.nass.usda.gov/).

[NASS] National Agricultural Statistics Service. 2010. Hawaii fruits annual summary. (http://www.nass.usda.gov/).

ALMEIDA, R. P. P., BENNETT, G. M., ANHALT, M. D., TSAI, C. W. & O'GRADY, P. 2009. Spread of an introduced vector-borne banana virus in Hawaii. *Molecular Ecology,* 18**,** 136-146.

ANDERSON, M. & BROMLEY, A. K. 1987. Sensory System. *In:* MINKS, A. K. & HARREWIJN, P. (eds.) *Aphids: Their Biology, Natural Enemies and Control.* Amsterdam: Elsevier Publishing Company.

ANHALT, M. D. & ALMEIDA, R. P. P. 2008. Effect of temperature, vector life stage, and plant access period on transmission of *Banana bunchy top virus* to banana. *Phytopathology,* 98**,** 743-748.

BENCHARKI, B., EL-YAMANI, M. & ZAOUI, D. 2000. Assessment of transmission ability of *Barley yellow dwarf virus*-PAV isolates by different populations of *Rhopalosiphum padi* and *Sitobion avenae*. *European Journal of Plant Pathology,* 106**,** 455-464.

BEREGOVOY, V. & PETERS, D. C. 1996. Efficiency of host plant use by eight biotypes of Schizaphis graminum (Aphididae: Homoptera). *Journal of the Kansas Entomological Society,* 69**,** 69-77.

BHADRA, P. & AGARWALA, B. K. 2010. A comparison of fitness characters of two host plant-based congeneric species of the banana aphid, *Pentalonia nigronervosa* and *P. caladii*. *Journal of Insect Science,* 10.

BHANOTAR, R. K. & GHOSH, L. K. 1969. On oviparous morph of *Pentalonia nigronervosa* Coquerel (Aphididae: Homoptera) from West Bengal, India. *Bulletin of Entomology,* 10**,** 97-99.

BLACKMAN, R. L. & EASTOP, V. F. 2000. *Aphids on the world's crops: An identification and information guide,* Chichester, John Wiley & Sons, Ltd.

BLACKMAN, R. L. & EASTOP, V. F. 2006. *Aphids on the world's herbaceous plants and shrubs,* London, John Wiley & Sons, Ltd.

BLACKMAN, R. L. & EASTOP, V. F. 2007. Taxonomic Issues. *In:* EMDEN, H. F. V. & HARRINGTON, R. (eds.) *Aphids as Crop Pests.* Wallingford, UK: CAB International.

BOQUEL, S., AMELINE, A. & GIORDANENGO, P. 2011. Assessing aphids potato virus Y-transmission efficiency: a new approach. *Journal of Virological Methods,* 178**,** 63-67.

BORTH, W., PEREZ, E., CHEAH, K., CHEN, Y., XIE, W. S., GASKILL, D., KHALIL, S., SETHER, D., MELTZER, M., WANG, M., MANSHARDT, R., GONSALVES, D. & HU, J. S. 2011. Transgenic banana plants resistant to Banana bunchy top virus infection. *Acta Horticulturae,* 31**,** 449.

BRAENDLE, C. & WEISSER, W. W. 2001. Variation in escape behavior of red and green clones of the pea aphid. *Journal of Insect Behavior,* 14**,** 497-509.

BRESSAN, A. & WATANABE, S. 2011. Immunofluorescence localisation of *Banana bunchy top virus* (family Nanoviridae) within the aphid vector, *Pentalonia nigronervosa*, suggests a virus tropism distinct from aphid-transmitted luteoviruses. *Virus Research,* 155**,** 520-525.

BRUNT, A. A., CRABTREE, K., DALLWITZ, M.J., GIBBS, A.J., WATSON, L. AND ZURCHER, E.J. (EDS.) 1996 Plant Viruses Online: Descriptions and Lists from the VIDE Database Version: 20th August 1996. http://biology.anu.edu.au/Groups/MES/vide/

CAREY, J. R. 1993. *Applied demography for biologists with special emphasis on insects,* New York, Oxford University Press, Inc.

CHARAABI, K., CARLETTO, J., CHAVIGNY, P., MARRAKCHI, M., MAKNI, M. & VANLERBERGHE-MASUTTI, F. 2008. Genotypic diversity of the cotton-melon aphid Aphis gossypii (Glover) in Tunisia is structured by host plants. *Bulletin of Entomological Research,* 98**,** 333-341.

CONANT, P. 1992. Banana bunchy top disease, a new threat to banana cultivation in Hawaii. *Proceedings of the Hawaiian Entomological Society,* 31**,** 91-95.

DALE, J. L. 1987. Banana bunchy top - An Economically Important Tropical-Plant-Virus Disease. *Advances in Virus Research,* 33**,** 301-325.

DALE, J. L., BURNS, T., OEHLSCHLAGER, S., KARAN, M. & HARDING, R. 1992. *Banana bunchy top virus*: Prospects for control through biotechnology. *Biotechnology Applications for Banana and Plantain Improvement.* San Jose, Costa Rica: (inibap) International Network for the Improvement of Banana and Plantain.

DALE, J. L. & HARDING, R. M. 1998. Banana bunchy top disease: current and future stratified for control. *In:* HADIDI, A., KHETARPAL, R. K. & KOGANEZAWA, H. (eds.) *Plant Virus Disease Control.* St. Paul, MN: APS Press.

DAVIS, J. A. & RADCLIFFE, E. B. 2008. The importance of an invasive aphid species in vectoring a persistently transmitted potato virus: *Aphis glycines* is a vector of *Potato leafroll virus*. *Plant Disease,* 92**,** 1515-1523.

DEDRYVER, C. A., RIAULT, G., TANGUY, S., GALLIC, J. F. L., TROTTET, M. & JACQUOT, E. 2005. Intra-specific variation and inheritance of BYDV-PAV transmission in the aphid *Sitobion avenae*. *European Journal of Plant Pathology,* 111**,** 341-354.

DEWAR, A. M., READ, L. A., HALLSWORTH, P. B. & SMITH, H. G. 1992. Effect of imidacloprid on transmission of viruses by aphids in sugar beet. 563-568.

DIXON, A. F. G. 1998. *Aphid Ecology An optimization approach,* London, Chapman & Hall.

EASTOP, V. F. 1966. A taxonomic study of Australian Aphidoidea (Homoptera). *Australian Journal of Zoology,* 14**,** 399-592.

EL-YAMANI, M. & BENCHARKI, B. 1997. Transmission of Moroccan isolates of *Barley yellow dwarf virus* by the Russian wheat aphid (*Diuraphis noxia*) and other aphid species. *Phytopathologia Mediterranea,* 36**,** 129-134.

FAUQUET, C. M., MAYO, M. A., MANILOFF, J., DESSELBERGER, U. & BALL, L. A. (eds.) 2005. *Virus Taxonomy: classification and nomenclature of viruses,* San Diego, CA: Elsevier Academic Press

FERERES, A. & MORENO, A. 2009. Behavioural aspects influencing plant virus transmission by homopteran insects. *Virus Research,* 141**,** 158-168.

FERRARI, J., DARBY, A. C., DANIELL, T. J., GODFRAY, H. C. J. & DOUGLAS, A. E. 2004. Linking the bacterial community in pea aphids with host-plant use and natural enemy resistance. *Ecological Entomology,* 29**,** 60-65.

FOOTTIT, R. G., MAW, H. E. L., PIKE, K. S. & MESSING, R. H. 2011. The aphids (Hemiptera: Aphididae and Adelgidae) of Hawai'i: Annotated list and key to species of an adventive fauna. *Pacific Science,* Early View.

FOOTTIT, R. G., MAW, H. E. L., PIKE, K. S. & MILLER, R. H. 2010. The identity of Pentalonia nigronervosa Coquerel and P. caladii van der Goot (Hemiptera: Aphididae) based on molecular and morphometric analysis. *Zootaxa***,** 25-38.

FUKATSU, T. 2010. A fungal past to insect color. *Science (Washington),* 328**,** 574-575.

GALAMBAO, M. B. 2011. *The population genetics of Pentalonia nigronervosa and P. caladii (Hemiptera: Aphididae) in Hawaii.* Master of Science Thesis, University of Hawaii.

GEERING, A. D. W. & THOMAS, J. E. 1997. Search for alternative hosts of banana bunchy top virus in Australia. *Australasian Plant Pathology,* 26**,** 250-254.

GIRM, M., WILDE, G. E. & REESE, J. C. 1992. Russian wheat aphid (Homoptera: Aphididae) feeding behavior on host and nonhost plants. *Journal of Economic Entomology,* 85**,** 395-401.

GOGGIN, F. L. 2007. Plant-aphid interactions: Molecular and ecological perspectives. *Current Opinion in Plant Biology,* 10**,** 399-408.

GOLD, C. S., PINESE, B. & PENA, J. E. 2002. Pests of Banana. *In:* PENA, J. (ed.) *Tropical Fruit Pests and Pollinators: Biology, Economic Importance, Natural Enemies and Control.* Wallingford, Oxon: GBR: CABI Publishing.

GRAY, S. & GILDOW, F. E. 2003. Luteovirus-aphid interactions. *Annual Review of Phytopathology,* 41**,** 539-566.

GRAY, S., M., POWER, A., G., SMITH, D., M., SEAMAN, A., J. & ALTMAN, N., S. 1991. Aphid transmission of *Barley yellow dwarf virus*: Acquistion access periods and virus concentration requirements. *Phytopathology,* 81**,** 539-545.

GRAY, S. M., SMITH, D. M., BARBIERRI, L. & BURD, J. 2002. Virus Transmission Phenotype Is Correlated with Host Adaptation Among Genetically Diverse Populations of the Aphid Schizaphis graminum. *Phytopathology,* 92**,** 970-975.

GRUNER, D. S. 2004. Arthropods From 'Öhi'a Lehua (Myrtaceae: Metrosideros polymorpha), With New Records for the Hawaiian Islands. *Records of the Hawaii Biological Survey for 2003. Bishop Museum Occasional Papers* 78**,** 33-52.

GUERRIERI, E. & DIGILIO, M. C. 2008. Aphid-plant interactions: a review. *Journal of Plant Interactions,* 3**,** 223 - 232.

HODGSON, D. J. 2001. Monoclonal aphid colonies and the measurement of clonal fitness. *Ecological Entomology,* 26**,** 444-448.

HOGENHOUT, S. A., AMMAR, E., WHITFIELD, A. E. & REDINBAUGH, M. G. 2008. Insect vector interactions with persistently transmitted viruses. *Annual Review of Phytopathology,* 46**,** 327-359.

HOOKS, C. R. R., FUKUDA, S., PEREZ, E. A., MANANDHAR, R., WANG, K.-H., WRIGHT, M. G. & ALMEIDA, R. P. P. 2009a. Aphid Transmission of Banana Bunchy Top Virus to Bananas After Treatment With a Bananacide. *Journal of Economic Entomology,* 102**,** 493-499.

HOOKS, C. R. R., MANANDHAR, R., PEREZ, E. P., WANG, K. H. & ALMEIDA, R. P. P. 2009b. Comparative Susceptibility of Two Banana Cultivars to Banana Bunchy Top Virus Under Laboratory and Field Environments. *Journal of Economic Entomology,* 102**,** 897-904.

HOOKS, C. R. R., WANG, K. H., PRADHAN, N. C., MANANDHAR, R., WRIGHT, M. G. & VORSINO, A. 2011. Population Distribution and Density of Pentalonia nigronervosa (Hemiptera: Aphididae) Within Banana Mats: Influence of Plant Age and Height on Sampling and Management. *Journal of Economic Entomology,* 104**,** 947-955

HOOKS, C. R. R., WRIGHT, M. G., KABASAWA, D. S., MANANDHAR, R. & ALMEIDA, R. P. P. 2008. Effect of banana bunchy top virus infection on morphology and growth characteristics of banana. *Annals of Applied Biology,* 153**,** 1-9.

HU, J. S., WANG, M., SETHER, D., XIE, W. & LEONHARDT, K. W. 1996. Use of polymerase chain reaction (PCR) to study transmission of *Banana bunchy top virus* by the banana aphid (*Pentalonia nigronervosa*). *Annals of Applied Biology,* 128**,** 55-64.

HU, J. S., XU, M. Q., WU, Z. C. & WANG, M. 1993. Detection of banana bunchy top virus in Hawaii. *Plant Disease,* 77**,** 952.

HULL, R. (ed.) 2002. *Matthew's Plant Virology,* Bath, UK: Academic Press.

JONES, D. R. (ed.) 2000. *Diseases of Banana, Abaca and Enset,* London, UK: CABI Publishing.

JONES, R. T., BRESSAN, A., GREENWELL, A. M. & FIERER, N. 2011. Bacterial Communities of Two Parthenogenetic Aphid Species Cocolonizing Two Host Plants across the Hawaiian Islands. *Applied and Environmental microbiology,* Vol 77**,** p. 8345-8349.

KEPLER, A. K. & RUST, F. G. (eds.) 2011. *The World of Bananas in Hawaii:Then and Now-Traditional Pacific & global varieties, cultures, ornamentals, health & recipes,* Haiku, Hawaii: Pali-O-Waipi'o Press.

KLINGAUF, F. A. 1987. Feeding, Adaptation and Excretion. *In:* MINKS, A. K. & HARREWIJN, P. (eds.) *Aphids: Their Biology, Natural Enemies and Control.* Amsterdam: Elsevier Science Publishers.

KUMAR, P. L., HANNA, R., ALABI, O. J., SOKO, M. M., OBEN, T. T., VANGU, G. H. P. & NAIDU, R. A. 2011. *Banana bunchy top virus* in sub-Saharan Africa: Investigations on virus distribution and diversity. 159**,** 171-182.

LINK, P. A.-L. & FUCHS, M. 2005. Transmission Specificity of Plant Viruses by Vectors. *Journal of Plant Pathology,* 87**,** 153-165.

LOMERIO, E. O. & CALILUNG, V. J. 1993. Comparative development of *Pentalonia nigronervosa* Coq. on five host plants. *Philippine Entomologist,* 9**,** 101-151.

LOWLES, A. J., TATCHELL, G. M., HARRINGTON, R. & CLARK, S. J. 1996. The effect of temperature and inoculation access period on the transmission of barley yellow dwarf virus by Rhopalosiphum padi (L.) and Sitobion avenae (F.). *Annals of Applied Biology,* 128**,** 45-53.

LOXDALE, H. D. 2008. The nature and reality of the aphid clone: genetic variation, adaptation and evolution. *Agricultural and Forest Entomology,* 10**,** 81-90.

MAGEE, C. J. P. 1927. Investigation on the bunchy top disease of the banana. *Commonwealth of Australia Council for Scientific and Industrial Research,* Melbourne, Australia.

MAGEE, C. J. P. 1940. Transmission studies on banana bunchy top *Journal of the Australian Institute of Agricultural Science***,** 109.

MANDAL, B., MANDAL, S., PUN, K. B. & VARMA, A. 2004. First report of the association of a Nanovirus with Foorkey disease of large cardamom in India. *Plant Disease,* 88**,** 428-428.

MARGARITOPOULOS, J. T., SHIGEHARA, T., TAKADA, H. & BLACKMAN, R. L. 2007. Host-related morphological variation within Myzus persicae group (Homoptera : Aphididae) from Japan. *Applied Entomology and Zoology,* 42**,** 329-335.

MEIRMANS, P. G. & VAN TIENDEREN, P. H. 2004. Genotype and Genodive: Two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes,* 4**,** 792-794.

MICHAUD, J. P., JYOTI, J. L. & QURESHI, J. A. 2006. Positive correlation of fitness with group size in two biotypes of Russian wheat aphid (Homoptera: Aphididae). *Journal of Economic Entomology,* 99**,** 1214-1224.

MORAN, N. A. & JARVIK, T. 2010. Lateral transfer of genes from fungi underlies carotenoid production in aphids. *Science (Washington),* 328**,** 624-627.

MURATORI, F. B., GAGNE, R. J. & MESSING, R. H. 2009. Ecological traits of a new aphid parasitoid, Endaphis fugitiva (Diptera: Cecidomyiidae), and its potential for biological control of the banana aphid, Pentalonia nigronervosa (Hemiptera: Aphididae). *Biological Control,* 50**,** 185-193.

NAJAR-RODRÍGUEZ, A. J., MCGRAW, E. A., HULL, C. D., MENSAH, R. K. & WALTER, G. H. 2009. The ecological differentiation of asexual lineages of cotton aphids: alate behaviour, sensory physiology, and differential host associations. *Biological Journal of the Linnean Society,* 97**,** 503-519.

NAULT, L. R. 1997. Arthropod transmission of plant viruses—a new synthesis. *Annals of the Entomological Society of America,* 90**,** 521-541

NELSON, S. C. 2006. *Banana Bunchy Top Disease in Hawaii* [Online]. College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa. Available: http://www.ctahr.hawaii.edu/banana/ [Accessed 2012].

NELSON, S. C., PLOETZ, R. C. & KEPLER, A. K. 2006. Musa species (banana and plantain). *In:* ELEVITCH, C. R. (ed.) *Traditional Trees of Pacific Islands-Their culture, environment, and use.* Holualoa, Hawaii: Permanent Agriculture Resources.

NELSON, S. C. & RICHARDSON, L. 2006. Banana bunchy top in Hawaii [videorecording]. Honolulu, HI: University of Hawaii at Manoa, College of Tropical Agriculture and Human Resources.

PADMALATHA, C. & RANJIT SINGH, A. J. A. 2001. Morphometric variations in Pentalonia nigronervosa Coq. (Homoptera : Aphididae) in relation to host plants. *Uttar Pradesh Journal of Zoology,* 21**,** 173-176.

PADMALATHA, C. & SINGH, A. J. A. R. 2001. Morphometric variations in *Pentalonia nigronervosa* Coq. (Homoptera:Aphididae) in relation to host plants. *Uttar Pradesh Journal of Zoology*, 21**,** 173-176.

PADMALATHA, C. & SINGH, A. J. A. R. 2002a. Life table and survivorship curve of Pentalonia nigronervosa Coq. (Homoptera : Aphididae). *Journal of Applied Zoological Researches,* 13**,** 156-159.

PADMALATHA, C. & SINGH, A. J. A. R. 2002b. Morphologically distinct forms of banana aphid, *Pentalonia nigronervosa* Coq. (Homoptera: Aphididae) in relation to host plants. *Journal of Applied Zoological Researches,* 13**,** 44-46.

PATEL, P. R. & SHUKLA, A. 2009. Studies on Host-Pathogen Interaction of Banana bunchy top. *International Journal of Plant Protection,* 2**,** 176-177.

PLOETZ, R. C., THOMAS, J. E. & SLABAUGH, W. R. 2003. Diseases of Banana and Plantain. *In:* PLOETZ, R. C. (ed.) *Diseases of Tropical and Subtropical Fruit Crops.* Wallingford, Oxon: GBR: CABI Publishing.

POPE, W. T. 1926 Banana culture in Hawaii. *United States Department of Agriculture,* Honolulu, Hawaii.

POWELL, G., TOSH, C. R. & HARDIE, J. 2006. HOST PLANT SELECTION BY APHIDS: Behavioral, Evolutionary, and Applied Perspectives. *Annual Review of Entomology,* 51**,** 309-330.

PRICE, P. W. 1984. *Insect ecology,* Wiley, New York.

RAJAN, P. 1981. Biology of *Pentalonia nigronervosa f. caladii* van der goot, vector of Katte disease of cardamom. *Journal of Plantation Crops,* 9**,** 34-41.

RAM, R. D. & SUMMANWAR, A. S. 1984. COLOCASIA-ESCULENTA (L) SCHOTT - A RESERVOIR OF BUNCHY TOP DISEASE OF BANANA. *Current Science,* 53**,** 145-146.

RAO, D. G. & NAIDU, R. 1974. Additional vectors of 'Katte' disease of small cardamom. *Indian Journal of Horticulture,* 31**,** 380-381.

ROBSON, J. D., WRIGHT, M. G. & ALMEIDA, R. P. P. 2007. Biology of *Pentalonia nigronervosa* (Hemiptera, Aphididae) on banana using different rearing methods. *Environmental Entomology,* 36**,** 46-52.

RUSSELL, J. A. & MORAN, N. A. 2006. Costs and benefits of symbiont infection in aphids: variation among symbionts and across temperatures. *Proceedings of the Royal Society of London. Series B, Biological Sciences,* 273**,** 603-610.

SADEGHI, E., DEDRYVER, C. A., RIAULT, G. & GAUTHIER, J. P. 1997. Variation in transmission of two BYDV-MAV isolates by multiple clones of *Rhopalosiphum padi* L. *European Journal of Plant Pathology,* 103**,** 515-519.

SIDDAPPAJI, C. & REDDY, D. N. R. N. 1972. A note on the occurrence of the aphid, Pentalonia nigronervosa form caladii Vander Goot (Aphididae-Homoptera), on cardamom (Elettaria cardamomom Salisb.). *Mysore Journal of Agricultural Sciences,* 6**,** 194-195.

SYLVESTER, E. S. 1980. Circulative and propagative virus transmission by aphids. *Annual Review of Entomology,* 25**,** 257-286.

SYMMES, E. J. & PERRING, T. M. 2007. Intraspecific variation in *Zucchini yellow mosaic virus* transmission by *Myzus persicae* and the impact of aphid host plant. *Journal of Economic Entomology,* 100**,** 1764-1772.

TASHIRO, H. 1967. Selfwatering acrylic cages for confining insects and mites on detached leaves. *J. Econ. Entomol.,* 60**,** 354-356.

THOMAS, J. E., ISKRA-CARUANA, M.-L. & JONES, D. R. 1994. Musa Disease Fact Sheet No. 4 Banana bunchy top disease. Montpellier, France: International Network for the Improvement of Banana and Plantain.

TIDONA, C. & DARAI, G. (eds.) 2011. *The Springer index of viruses,* New York: Springer Science + Business Media.

TSUCHIDA, T., KOGA, R., HORIKAWA, M., TSUNODA, T., MAOKA, T., MATSUMOTO, S., SIMON, J. C. & FUKATSU, T. 2010. Symbiotic bacterium modifies aphid body color. *Science (Washington),* 330**,** 1102-1104.

VARMA, P. M. & CAPOOR, S. P. 1958. Mosaic disease of cardamom and its transmission by the banana aphid Pentalonia nigronervosa Coq. *Indian Journal of Agricultural Sciences,* 28**,** 97-107.

VENUGOPAL, M. N. 1995. Viral diseases of cardamom (*Elettaria cardamomum* Maton) and their management. *Journal of Spices and Aromatic Crops,* 4**,** 32-39.

VENUGOPAL, M. N., MATHEW, M. J. & ANANDARAJ, M. 1999. Breeding potential of *Pentalonia nigronervosa f. caladii* Van der Goot (Homoptera: Aphididae) on katte escape cardamom (Elettaria cardamomum Maton) plants. *Journal of Spices and Aromatic Crops,* 8**,** 189-191.

VISWANATHAN, S. & REGUPATHY, A. 1992. Bioecology of banana aphid *Pentalonia nigronervosa f. typica*. 42-49.

WAGNER, W. L., HERBST, D. R. & SOHMER, S. H. (eds.) 1999. *Manual of the Flowering Plants of Hawaii,* Honolulu: University of Hawai'i Press, Bishop Museum Press.

WARDLAW, C. W. 1972. The Virus Diseases: Bunchy Top. *Banana Diseases including Plantains and Abaca.* 2nd ed. London, UK: Longman.

WATERHOUSE, D. F. 1987. Chapter 6. *Pentalonia nigronervosa* Coquerel. *In:* WATERHOUSE, D. F. & NORRIS, K. R. (eds.) *Biological Control: Pacific Prospects.* Inkata Press, Melbourne.

WILL, T. & VAN BEL, A. J. E. 2006. Physical and chemical interactions between aphids and plants. *J Exp Bot,* 57**,** 729-737.

WILLIAMS, I. & DIXON, A. F. G. 2007. Life Cycles and Polymorphism. *In:* VAN EMDEN, H. F. H., R. (ed.) *Aphids as Crop Pests.* Wallingford, UK ; Cambridge, MA: CABI.

WOODFORD, J. A. T. 1992. Effects of systemic applications of imidacloprid on the feeding behaviour and survival of *Myzus persicae* on potatoes and on transmission of *Potato leafroll virus*. *Pflanzenschutz-Nachrichten Bayer (English ed.),* 45**,** 527-546.

WU, R. Y. & SU, H. J. 1990a. Production of monoclonal-antibodies against *Banana bunchy top virus* and their use in enzyme-linked-immunosorbbent-assay. *Journal of Phytopathology-Phytopathologische Zeitschrift,* 128**,** 203-208.

WU, R. Y. & SU, H. J. 1990b. Transmission of *Banana bunchy top virus* by Aphids to Banana Plantlets from Tissue-Culture. *Botanical Bulletin of Academia Sinica,* 31**,** 7-10.

XIE, W. S. & HU, J. S. 1995. Molecular-cloning, sequence-analysis, and detection of *Banana bunchy top virus* in Hawaii. *Phytopathology,* 85**,** 339-347.

YASMIN, T., KHALID, S., SOOMRO, M. H., MALIK, S. A., SHAH, H. & AHMAD, I. 2001. Specificity of Host-Pathogen Interaction of Banana bunchy top Disease. *Online Journal of Biological Sciences,* 1**,** 212-213.