Determining fruit fly (Family Tephritidae) host status of moringa tree pods, Moringa oleifera Lam., in Hawai'i

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Introduction

The moringa tree, *Moringa oleifera* Lam., is in the Family Moringaceae, which includes 14 species (Emongor 2011). Some other common names for M. oleifera include drumstick tree, horseradish tree, marrunggai, malunggai, and kalamunggai (Radovich 2009). The moringa tree is native to Northern India, Pakistan, Bangladesh, and Nepal, and this tree is cultivated in South Asia, Southeast Asia, the Arabian Peninsula, tropical Africa, Central America, the Caribbean, and tropical South America (Morton 1991, Parrotta 2009). Moringa oleifera is a tree that grows 10 to 12 meters high (Fig. 1A), and this tree is found from sea level to 1400 meters in its native range (Parrotta 2009). The fruit is a three-sided pod with nine longitudinal ridges that are about 20 to 50 cm long and 2.0 to 2.5 cm wide (Parrotta 2009), and some varieties have pods up to 120 cm long (Ramachandran et al. 1980). Immature pods are green (Fig. 1B) or reddish, and mature pods are brown (Fig. 1C) with 15-20 large brown to black seeds with three papery wings (Radovich 2009). Pods mature three months after flowering (Parrotta 2009), and the seeds germinate in two weeks (Radovich 2009). Moringa starts flowering four to twelve months after planting (Radovich 2009), and a single tree in India can yield up to 600 pods in a year with two crop seasons (Ramachandran et al. 1980).

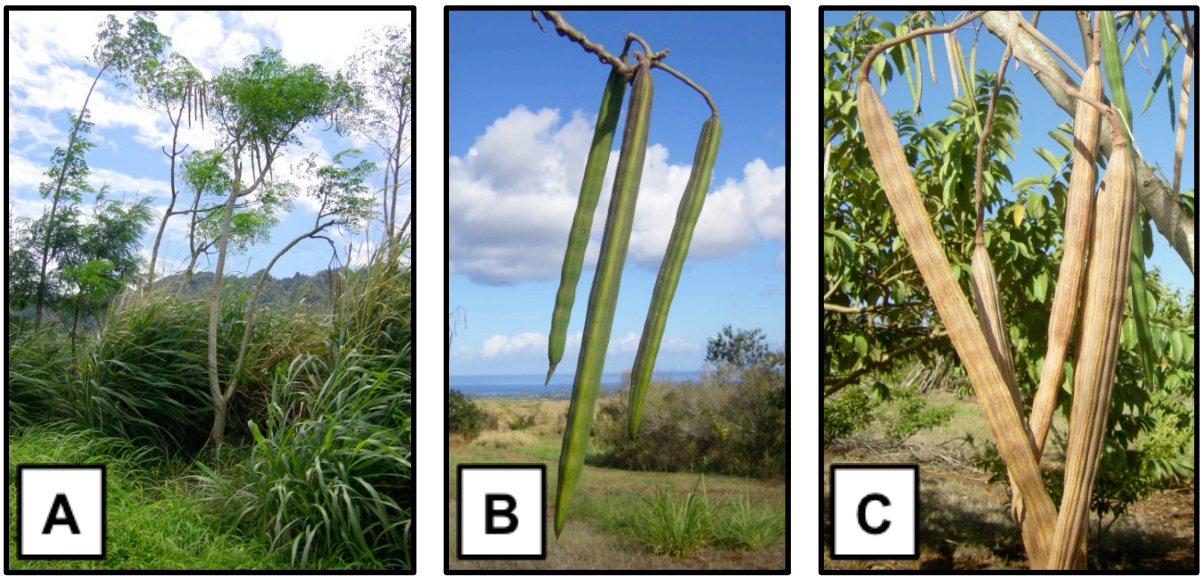


***Figure 1**. A) Moringa tree, B) Green moringa seed pods, and C) Mature moringa seed pods*

Moringa oleifera is adapted to living in the tropics and subtropics, and high average temperatures of 25-30 °C, well distributed annual rainfall of 1000 to 2000 mm, high solar radiation, and well-drained soils are all requirements for optimum leaf and pod production in moringa trees (Radovich 2009). Moringa grows best below 600 meters, and it tolerates a wide range of soil types and pH (4.5-9) but grows best in well-drained soil with neutral pH (Radovich 2009). Mor-

inga trees are tolerant of drought and poor soils, and these trees respond well to irrigation and fertilization (Radovich 2009). Bees and birds pollinate moringa trees (Morton 1991), and trees will produce good seed crops for about twelve years (Parrotta 2009). Moringa seeds can be directly planted, (Radovich 2009), or moringa can also be propagated by cuttings, which produce flowers and fruit more quickly than growing plants by seed (Parrotta 2009). Flowering and fruit production happens throughout the year in areas such as Florida, Cuba, and Jamaica that have constant seasonal temperatures and rainfall (Morton 1991, Parrotta 2009), and Hawai'i also has year round pod production. The best yields are from warm and dry conditions with some supplemental fertilizer and irrigation (Radovich 2009). PKM-1 and PKM-2 are popular early flowering varieties, and Bombay, Chavakacheri, Chemmurungai, and Jaffna are other named varieties (Radovich 2009, Ramachandran et al. 1980). Moringa trees are common on commercial farms and in backyards throughout the island of O'ahu.

Moringa consumption and products

Fresh moringa products are often eaten within one to two days of harvest, and moringa pods can be canned to preserve them for later consumption (Radovich 2009). Young pods are cooked like string beans, and larger pods are pickled or used in soups, stews, and curries (Radovich 2009, Ramachandran et al. 1980). Moringa leaves are highly nutritious, and fresh or powdered leaves are used to combat malnutrition in India and other developing nations (Radovich 2009, Rahim et al. 2007, Pandey et al. 2011). Leaves are high in protein content (27%), vitamins A and C, beta carotene, potassium, calcium, iron and phosphorus, and they are also a good source of natural antioxidants (Gupta et al. 1989, Dillard and German 2000, Pandey et al. 2011). Flowers and leaves are also eaten as salad or are cooked for use in soups and sauces, and fried seeds are consumed and taste like peanuts (Ramachandran et al. 1980, Emongor 2011). The leaves and flowers can also be used for tea, and Zija ™ is a commercial health food drink that contains 30 grams of moringa, leaf, seed, and pod (Radovich 2009). Cooking oil is also extracted from the seeds, and it is considered high grade oil similar to olive oil (Emongor 2011). Moringa oil can be stored for years without a reduction in oil quality, and leaves in plastic bags and pods can be stored at 10 °C for 5-14 days (Foidl and Paull 2008).

The moringa tree has many other uses besides food (Emongor 2011). The seed cake that is left over from extracting seed oil can be used as a fertilizer (Radovich 2009). The oil from moringa tree seeds is used in the cosmetic industry, illuminant, lubricant for watches, and a biofuel (Pandey et al. 2011, Rashid et al. 2008, Parrotta 2009). Reddish-brown to brownish black gum from the stem of the tree is used for dye in calico printing, which is the process of making fast-color designs on cotton fabrics (Pandey et al. 2011, Ramachandran et al. 1980). Moringa trees can also be used as live fence posts, and leaf extracts are used for crop plant growth promotion (Radovich 2009). Leaves and twigs are used as food for cattle, sheep, goats, camels, pigs, chickens, fish and rabbits (Radovich 2009, Parrotta 2009, Mahatab et al. 1987, Negi 1977), and flowers are a good source of pollen for honeybees (Parrotta 2009). A poultice of moringa leaves and bark are directly applied to wounds as an anti-microbial and to promote

healing (Radovich 2009). Pterogospermin, an antibiotic that is effective for the treatment of cholera, is found in the roots and flowers, and juice extracted from the leaves has antimalarial and antibacterial properties (Parrotta 2009).

Arthropod pests of moringa trees

Moringa has many insect pests in its native range, and most of the literature is from India. Eight Lepidoptera species, four Hemiptera species, one Coleoptera stem borer, and one Diptera species have been identified as pest species in India (Sivagami and David 1968, Ramachandran et al. 1980, Parrotta 2009). The Diptera on moringa is in the Family Drosophilidae and genus *Gitonia* sp. (Parrotta 2009), and no tephritid fruit flies have been identified as a pest of moringa in any literature from the native range or cultivated regions of the world. In Puerto Rico, moringa trees are highly susceptible to attack by termites, and heavy seed predation by an unidentified insect was often found (Parrotta 2009). Little information is available about moringa insect pests in Hawai'i, and mites are the largest economic pest for leaves because of the possible rejection of shipments to North America (Radovich 2009). Usually arthropod pests on moringa trees do not require pesticide treatment, but neem oil, horticultural soap and sulfur can control mites, aphids and other pests (Radovich 2009). Only mites, aphids, imported cabbageworm (*Pieris rapae*) and borers causing trunk damage have been observed on moringa (Radovich 2009). For this reason, it is important to identify other insect pest species on moringa to determine if quarantine treatment is necessary.

Fruit flies are economically important pests that have been found in Hawai'i since 1895, and they are found from sea level to above 2134 meters (Messing 1999). Tephritid fruit flies lay clutches of eggs under the skin of fruit and vegetables, and larvae feed internally on the pulp, which makes visual inspection for infestations very difficult (Arthur et al. 2009, Messing 1999). There are federal quarantine regulations for the movement of all fruits and vegetables from Hawai'i because of four invasive tephritid fruit flies, which include *Ceratitis capitata* (Wiedemann) (Mediterranean fruit fly), *Bactrocera dorsalis* (Hendel) (oriental fruit fly), *B. cucurbitae* Coquillett (melon fly), and *B. latifrons* (Hendel) (solanaceous fruit fly) (Follett and Armstrong 2004, Arthur et al. 2009). Currently, moringa leaves are shipped to the mainland USA, but moringa pods must be irradiated at 150 Grays (Gy) for tephritid fruit flies in Hawai'i or upon entry into the mainland USA states with irradiation facilities (USDA 2013, USDA-APHIS 2006). A preharvest broad spectrum insecticide or a post-harvest dip is also required with irradiation unless treated at 400 Gy dose (USDA 2013). Irradiation treatment is expensive for growers, so they only currently ship moringa pods to Canada or sell them at local farmers markets. On the island of O'ahu, many growers that produce herbs, taro, or eggplant also have moringa trees, and they ship the leaves by plane to the west coast of the mainland USA or sell pods at local farmers markets.

In collaboration with Dr. Nicanor Liquido from USDA APHIS PPQ - CPHST, Plant Epidemiology and Risk Analysis Laboratory, we are interested in determining if moringa pods are a suitable

host for tephritid fruit fly species in Hawai'i, which are Ceratitis capitata, Bactrocera dorsalis, B. cucurbitae, and B. latifrons (Follett and Armstrong 2004, Arthur et al. 2009). All four of these fruit fly species feed on a wide variety of fruit and vegetable hosts (Messing 1999). For this reason, it is important to identify other insect pest species on moringa to determine if quarantine treatment is necessary. Moringa pods have identified as a host for Drosophilidae in the genus Gitonia (Parrotta 2009), but there is no information about tephritid fruit flies in moringa pods. We investigated moringa pods as a host for C. capitata, B. dorsalis, B. cucurbitae, and B. latifrons. This study will help us determine if moringa pods need quarantine treatment for tephritid fruit flies.

Sampling moringa pods for fruit flies

Most of the commercial moringa production is located on the island of O'ahu, and this was the main area for our survey. In September 2013, we met with moringa growers on O'ahu that had trees with pods and established contacts for future sampling sites. Sampling sites at 5 commercial farms and 2 University of Hawai'i (UH) research stations were established in October 2013. There were three different locations for the 5 commercial farms, which included Ewa Beach (1 farm), Waialua (1 farm), and Kahuku (3 farms). The two UH research stations in this study were the Poamoho Research Station near Waialua and Waimānalo Research Station in Waimānalo. There was also one farm on the island of Moloka'i in Ho'olehua. The number of moringa trees on farms ranged from 5 to 500, so a sampling plan was established to accommodate different plot sizes. For small (5-20) groups of trees, every third tree was sampled, and every fifth tree was sampled for plots with more than 20 trees or large plots. One farm in Kahuku, 1 farm in Ewa Beach, UH Poamoho, UH Waimānalo locations were considered large plots, and 2 farms in Kahuku, 1 farm in Waialua, and 1 farm in Moloka'i were designated small groups.

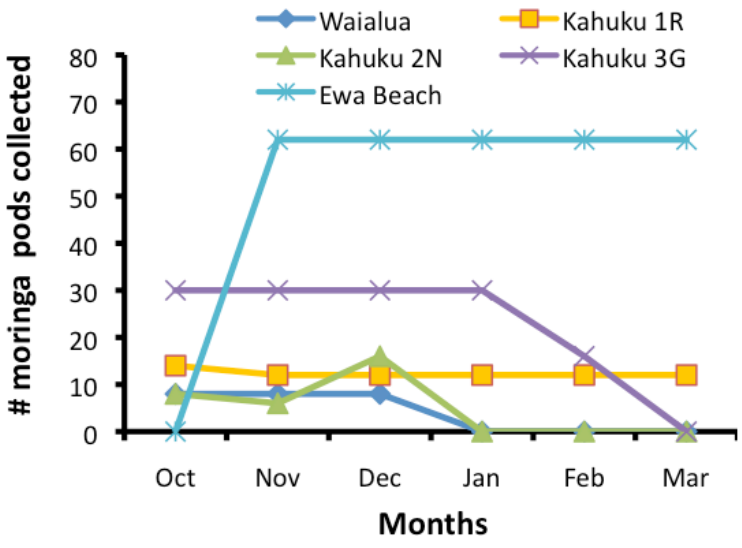
Two mature green pods were removed from each tree sampled, and these pods were placed in paper bags and were brought back to the laboratory. The pods were placed in clear plexiglass tube cages that were 30.5 cm long by 15.2 cm wide with opaque white organdy mesh squares that were 26 cm by 26 cm (Time chiffon, Walmart) secured on the tube cages with Advantage file bands (17.78 x 0.31 cm rubber bands, Walmart) on both sides and were placed on wire metal shelving units that were four shelves high. Five to seven pods were placed in each tube cage. Three (40 cm long, 30 cm wide and 30 cm high) rectangular plexiglass cages with were also used if more room was needed to hold pods for each month. All of the cages were kept in the laboratory at 22.2 °C, and the pods were observed for insect emergence for 30 days. The cages were checked for insect emergence two to three times a week. Samples were collected monthly from October 2013 to March 2014 at farms on O'ahu, and one sample was collected from the Moloka'i farm in January 2014. The date of insect emergence and original pod location along with the date were recorded, and the insects were stored in labeled petri dishes or condiment cups in the laboratory freezer until identified.

Pods were removed from all 88 trees at the University of Hawai'i Poamoho research station using a pole trimmer or hand saw, and approximately 2500 moringa pods from 52 trees were held in the laboratory at 22.2 °C for insect emergence. Pods were separated into three groups, which were young green pods, mature green pods, and brown seed pods. Insects were collected from cages and were identified. Forty-eight moringa trees at Poamoho were marked with flagging tape for monthly mature pod sampling. Pods from both University of Hawai'i research stations were also dissected for insect larvae with 90 mature pods from Poamoho and 30 mature pods from Waimānalo for 120 total pods. Forty moringa pods from Moloka'i were also dissected for insect larvae.

Results of moringa pod sampling

All moringa pods sampled in this study were mature green pods, which are edible for consumption, and this is the stage before they become brown seed pods. Older mature green pods begin to turn gray or light brown as the seeds are forming inside the pod and are not considered edible because the pod starts to harden and loses moisture. For the moringa pod sampling at commercial farms on the island of O'ahu, 741 total pods were collected and observed for insect emergence in the laboratory at 22.2 °C. These pods were collected from five different commercial farms from October 2013 to March 2014. There was no October 2013 collection for the Ewa Beach location (Fig. 2), but there were moringa pods collections from this location for the next 5 months of sampling. In October 2013, 103 moringa pods were collected at 1 commercial farm in Waialua and 3 commercial farms in Kahuku (Fig. 2). For November 2013 through March 2014, the five commercial sampling sites were Ewa Beach (1 farm), Waialua (1 farm), and Kahuku (3 farms). The total number of the moringa pods collected for each month was 118 pods for November 2013, 128 pods for December 2013, 104 pods for January 2014, 90 pods for February 2014, and 74 pods for March 2014. The moringa pod production in winter did decrease, and three of the farms trimmed their moringa trees, which led to no pods from those locations (Fig. 2). The farm in Waialua and one farm in Kahuku (2N) trimmed back their trees in late December, and 1 more Kahuku farm (3G) trimmed their trees in February, which led to no pods for collection in March 2014 (Fig. 2). Twenty pods

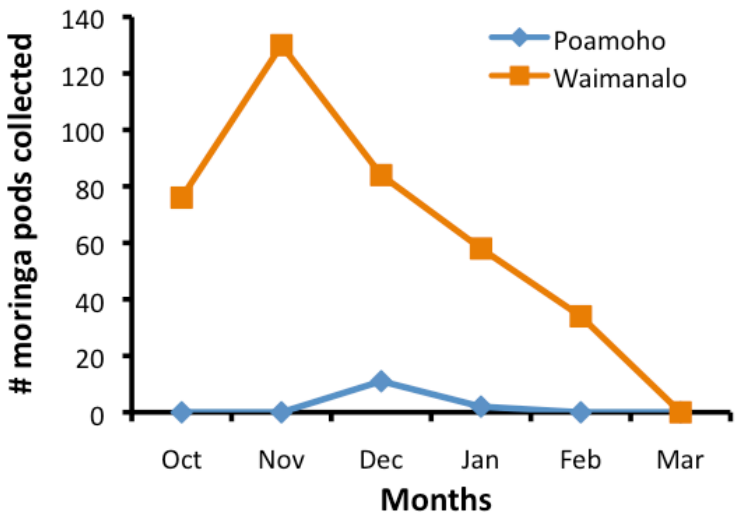
Figure 2. *The number of moringa pods collected at five commercial farms on O'ahu from October 2013 to March 2014.*



were also collected from the farm on the island of Moloka'i and were also put into tube cages.

The two UH research stations in this study were Poamoho Research Station and Waimānalo Research Station, and they were sampled from October 2013 to March 2014 (Fig. 3). All of the old pods were removed from the Poamoho location in October 2013, so pods were not available for sampling until December 2013 (Fig. 3). Only 11 moringa pods in December and 2 pods in January were available for sampling at Poamoho because the trees were trimmed. Waimānalo pod collection also decreased over time with the lowest pod collection in February, and there were no available pods in March (2014). The total number of moringa pods for each month was 94 pods for October 2013, 130 pods for November 2013, 95 pods for December 2013, 60 pods for January 2014, 34 pods for February 2014, and zero pods for March 2014. The Waimānalo research station also showed the same decline in pod production as the commercial farms (Fig. 3).

Figure 3. *The number of moringa pods collected at two University of Hawai'i farms on O'ahu from October 2013 to March 2014.*



The insects that emerged out of moringa pods were labeled, recorded and placed in the freezer for later identification. For the five commercial farms, insects were only found at three of the 5 farms (Table 1). There were only 56 insects collected over the 6 month study, and the insects found were in only four Orders: Psocoptera, Hemiptera, Coleoptera, and Diptera. These insects did not emerge from the pods until after the pods were moldy or had become brown seed pods at three to 4 weeks after field collection. More insects were found emerging from pods at the Waimānalo research station than at all 5 of the commercial farms (Table 2) with 229 total insects collected from the tube cages over 6 months. Different species in the orders Coleoptera, Lepidoptera, and Diptera were found at this site than the commercial farms. This could be due to different management strategies. The Waimānalo farm was not managed for weeds or high plant No insects emerged from the 13 pods at Poamoho, but insects did emerge from the 2500 pods collected in October 2013 (Table 3). Other insects were also found when old seed pods from the ground and small pieces of dead branches were collected (Table 3). No tephritid fruit flies emerged from any of the 3487 pods that were held in the laboratory, even though they were seen landing or roosting on moringa leaves. Only one beetle larva was found in one pod from Poamoho, and no fruit fly larvae or oviposition holes were discovered on any dissected pods. No larvae were found in the 40 moringa pods from farm on Moloka'i.

Table 1. *Total number of insects that emerged from moringa pods in tube cages held at 22.2 °C in the laboratory for 30 days. Moringa pods were collected at five commercial farms on O'ahu from October 2013 to March 2014.*

Farm	Location	Order	Family	Subfamily	Genus species	Total # collected
Fat Law	Ewa Bch	Psocoptera	Unknown			5
	Ewa Bch	Psocoptera nymph	Unknown			1
Gao	Kahuku	Hemiptera	Miridae			1
Rod	Waialua	Coleoptera	Cuculionidae	Scolytinae		7
Rod	Waialua	Diptera	Phoridae			40
	Waialua	Diptera	Ottitidae		*Notogramma cimicforme*	1
Rod	Waialua	Diptera	unknown			4
Rod	Waialua	Diptera pupae	unknown			2
Gao	Kahuku	Diptera	unknown			2

Table 2. *Total number of insects that emerged from moringa pods in tube cages held at 22.2 °C in the laboratory for 30 days. Moringa pods were collected at two University of Hawai'i (UH) farms on O'ahu from October 2013 to March 2014. No insects emerged from the UH Poamoho farm.*

Location	Order	Family	Subfamily	Genus species	Total # collected
Waimanalo	Psocoptera	unknown			6
	Coleoptera	Curculionidae	Scolytinae		1
		Nitidulidae larvae			12
	Lepidoptera	Crambidae			2
		Unknown			2
		Unknown larvae			4
		Unknown pupa			1
	Diptera	Phoridae			3
		Chloropidae		*Gampsocera hardyi*	4
		Neriidae		*Telostylinus lineolatus*	5
		Muscidae		*Atherigona orientalis*	22
		Unknown larvae			2
		Unknown pupa			1
	Unknown pupae				164

Table 3. Total number of insects that emerged from moringa pods in tube cages held at 22.2 °C in the laboratory for 30 days. All of the moringa pods were removed from 88 trees at University of Hawai'i (UH) research station farm on O'ahu in October 2013, and a subset of the total number of pods were held in tube and plexiglass cages. Other insect species associated with moringa trees were captured during this time period.

Order	Family	Genus	Part of moringa tree	Total # collected
Blattodea	Unknown nymph		Old seed pods	2
Dermaptera	Labiidae		Old seed pods	2
Coleoptera	Cerambycidae		Old seed pods/branches	5
	Curculionidae		Old seed pods	2
	Nitidulidae	*Carpophilus* sp.	Old pods/old seed pods	193
	Nitidulidae larvae			19
Lepidoptera	Unknown		Old seed pods	2
Hymenoptera	Tiphiidae		Moringa flowers	1

Conclusions

There has been a growing interest on O'ahu to find more ways to market and sell different edible parts of the moringa, since many growers already have moringa trees on their farms. Moringa trees are underutilized in the Pacific area and Hawai'i, and leaves and pods of this tree are mainly used for consumption by South and Southeastern Asian families (Radovich 2009). Increasing awareness and appreciation of moringa trees beyond current cultural boundaries will provide a sustainable, local food source that will enhance nutrition and food security in Hawai'i and other island communities in the Pacific (Radovich 2009). The optimization of moringa oil production for use as cooking oil and possibly bio-diesel could improve grower profitability and help enhance community self-sufficiency as well (Radovich 2009).

This study has helped prove that moringa pods are not a suitable host for the four invasive fruit flies found in Hawai'i. No tephritid fruit flies were found emerging from any of the moringa pods that were collected from commercial farms or research stations. All of the insects that emerged from the pods were found long after the pods would be marketable or edible, and these insects are predominantly detritivores that fed on decaying matter or fungus that grew on the pods. One alternative to postharvest treatment is nonhost status, which means that a commodity is proven to be a not a fruit fly host for all or part of its growth cycle (Follett and Neven 2006). A fruit fly host defined as fruit or vegetable onto which an insect deposits eggs, the eggs hatch into larvae and larvae feed and develop to form viable adults (Armstrong 1986, Armstrong 1994). If insect cannot completely develop to form viable adults then plant is nonhost (Follett

and Neven 2006). More laboratory host testing can be done to determine if female fruit flies would try to oviposit in moringa pods, but the preliminary sampling indicates that moringa is not used by tephritid fruit flies as a host. Moringa pods are a good candidate for a change in quarantine regulation that currently includes irradiation treatment for export from Hawai'i. Removing the irradiation treatment for export would allow local growers to sell and ship fresh pods to the west coast of mainland U. S., which would improve rural grower profitability for currently unused moringa pods on trees. Dr. Liquido has begun the necessary processes in the USDA to change the current regulations on moringa export.

Acknowledgements

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USDA APHIS. 2013. Reference. Hawaii. Regulation and clearance from Hawaii to other parts of the United States Manual. Pp. 21-28. (Table 3-1 List of approved fresh fruits, herbs, and vegetables from Hawaii-Authority 7CFR 318.13. http://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/hawaii.pdf.

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