Lemon (*Citrus limon*) as a host for Mediterranean fruit fly (Medfly; *Ceratitis capitata*)

A scientific review and status report

USDA APHIS and USDA ARS

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I. Introduction and Background

This report presents findings from a meeting of USDA-APHIS fruit fly regulatory and trade personnel and Agricultural Research Service (ARS) fruit fly experts held September 12 and 13, 2007, at PPQ headquarters in Riverdale.

The purpose of this meeting was to address the status of lemons as hosts for Mediterranean fruit fly.

In preparation for the meeting, the participants were sent copies of the papers in the references, an analysis of those papers which is presented here as Table 1, and a copy a research protocol for determining host status of lemons to Medfly proposed to be conducted cooperatively in Spain,.

In the past, APHIS has not considered lemons, *Citrus limon*, to be a host for Medfly. Consequently, no phytosanitary requirements for Medfly were imposed on imported lemons. A recent 2006 incident where live Medfly larvae were intercepted in Spanish lemons has cast doubts on our current regulatory policy for the movement of lemons from Medfly infested areas into free areas of the United States either during domestic outbreaks or as foreign imports. The detection of Medfly larvae in lemons from Spain required APHIS to take emergency action to require Verna lemons from Spain to be cold treated. In addition, Argentina has proposed to export lemon to the US and the occurrence of fruit flies in the citrus growing areas poses a concern. Domestically, periodic outbreaks of Medfly in the US (e.g. Florida and/or California) will mean that regulations or revised policies will have implications for domestic producers of lemons.

We found that the evidence from port interceptions, plans for new research in Spain regarding lemons as Medfly hosts, the re-launching of the Argentine lemon risk assessment and recent lemon host studies conducted by ARS all justified a comprehensive internal review and discussion of the technical facts and regulatory dynamics.

As per our CFR, the current regulated article list in the domestic Medfly quarantine, 7 CFR 301.78-2, lists lemons as follows:

"Lemon (*Citrus limon*) except smooth-skinned lemons harvested for packing by commercial packing houses."

That is, "smooth-skinned lemons harvested for packing by commercial packing houses" are not currently regulated. The decision to list lemons with the above exception was based, in part, on research published by ARS scientists (Spitler et al. 1984, see references below).

However, since the original reports that led to the current regulatory status, there have continued to be investigations into lemons and fruit fly dynamics. Experiments by ARS (2000, internal report) include findings that harvested yellow lemons are a suitable fruit fly host. It has become apparent that these and other recent observations may limit the applications of the findings of the previous published scientific article (Spitler et al. 1984, and similar).

In terms of regulatory impacts domestically, changes to the host status of lemons would impact:

- The exports and domestic movement of both California and Florida lemons during a Medfly outbreak situation.
- Entry requirements of Spanish lemons
- Potential entry requirements for Argentine lemons.
- Entry requirements from any area where fruit flies occur that wish to export lemons to the US

II. Objectives

The meeting began with a discussion of general principles of resistance mechanisms and the evolving nature of the concept of host/non-host, recognizing that the clear distinction is increasingly giving way to 'conditional host' concepts.

Despite the broad discussions, the meeting focused on these specific issues:

Designate conditions that determine whether/when lemons are a host and a pathway for introduction, including addressing the following:

- At what stage of maturity or ripeness do lemons become susceptible--both on the tree and off the tree?
- Are there differences in the host status of varieties?
- Are there any needed changes to current regulatory policy?
- Is more research needed to answer these questions and, if so, what protocols should be followed and where should it be done?

III. Trade Issues

Spain. Spain is one of the largest exporters of lemons in the world (other major exporters include Argentina and Turkey). There are two main varieties of lemons imported from Spain: Fino and Verna. The late-season Verna lemons were the only variety found to be infested. Verna lemons are harvested and shipped yellow and Fino harvested green, de-greened with ethylene, and shipped yellow. Current (emergency) requirements for cold treatment following schedule T107a apply only to Verna lemons; evidence indicates that Fino lemons do not currently pose a risk for Medfly. As a result of the cost of treatment being too high and phytotoxicity constraints, Spain is not currently exporting Verna lemons. Fino lemons are shipped with a phytosanitary certificate with an additional declaration stating that they are harvested green. Finos are harvested from October to March, which is the late fall-winter-early spring season in Spain. Vernas are harvested from January to August which is the winter-summer season in Spain.

Argentina. Argentina has petitioned APHIS for market access for lemons. A PRA has been completed and PPQ has conducted a site visit to Argentina in August, 2007, to observe lemon production, harvesting, and packing practices and fruit fly research. Lemons produced for export in Argentina include Lisbon, Eureka, Fino, Genoa and Limoneiro 8A varieties. These lemons are shipped between April and September, which is the fall-winter season in Argentina.

Other. Any country that wishes to export lemons and has tephritid fruit flies is likely to be a concern at this time.

IV. Site Visits to Spain and Argentina

Spain

A site visit by APHIS to Spain in Sept. 2006, found that there are numerous abandoned orchards in the production area. Trapping is not required in the lemon growing areas, but trap observations from adjacent Clementine growing areas indicated very high population levels of Medfly. Medfly control is not required until just before the Clementine season (October), which is after the Verna lemon season, allowing Medfly populations to increase over summer months. Traps were relatively sparsely distributed in Clementine production areas. Differences in susceptibility in varieties (Verna compared to Fino) are most likely due to seasonal differences in Medfly population levels, maturity of the lemons during those seasons, that Fino is harvested green and Verna yellow, and availability of other more susceptible or preferred hosts, rather than differences in resistance mechanisms in different varieties of lemons.

Argentina

A site visit to Argentina by CPHST and PPQ staff included visits to production areas and packing houses. Eduardo Willink, Director of Disciplinas Especiales, Estacion Experimental Agroindustrial Obispo Columbres, Tucuman, through SENASA, provided data from extensive experiments conducted under field and under laboratory conditions, and from sampling fruit in the field and in packing houses. He concluded that under conditions in Argentina, lemon is not a host of Medfly. Medfly occurs at low levels in the production areas, and lemon is harvested for export during fall-winter months. APHIS is currently continuing to review the data provided by Argentina.

The packing houses use a computer based sorting system. Acceptable fruit for export to the EU and Canada are those that are light yellow in color which also conform to diameter specifications. The light yellow color fruit is exported because it maintains its shelf life longer than yellow fruit. The sorting system also automatically culls fruit that is misshapen or blemished.

V. Conclusions based on available scientific evidence

Over 90 publications and reports were reviewed and analyzed to provide supporting evidence with regard to the host status of lemon. Table 1 provides a summary and concise analysis of each of the publications.

Based the analytical review of the publications, the evidence supports the following conclusions:

- Green lemons are not hosts of Medfly but susceptibility to infestation increases as lemons mature;
- Host susceptibility rises markedly once the fruit is harvested, with noticeable changes (increased susceptibility) detectable within 24 hours.
- Resistance to Medfly is causally linked to:
  - Chemical toxicity in rind;
  - Rind thickness and toughness;
- High population pressure increases likelihood of infestation;
  - repeated oviposition by females into an existing oviposition puncture hole can overcome physical barriers
- Over-mature lemons (lemons left on trees past the normal harvest) are more susceptible due to:
  - Changes in rind chemistry and reduced rind thickness;
  - Longer time that the fruit is subject to repeated oviposition by females into existing puncture holes can overcome physical barriers
- Drought conditions or other stresses on hosts can affect susceptibility.
- Medfly larvae can develop within the pulp. Medfly anatomy is such that its ovipositor cannot pierce through the rind of the fruit. However, if the rind is damaged or existing oviposition puncture holes are present, females can exploit the damage or holes by ovipositing into them and the Medfly eggs and larvae will be more likely to survive and develop.
- Medfly will attack less susceptible hosts if more susceptible or preferred hosts are not available.
- Fruit from the ground is likely to be over-mature, over-ripe, or damaged, and therefore highly likely to be infested.

It was the consensus of the expert team that lemon fruit is not a host for Medfly while still green (not fully mature). It is likely that light yellow lemons are not at a maturity stage where they would be susceptible to Medfly.

Are harvested lemons a host and, if so, at what stage of ripeness or maturity?

Yes, harvested lemons can be Medfly hosts. Susceptibility increases over time post harvest, especially for mature lemons.

Are lemons on the tree a host and, if so, at what stage of ripeness or maturity?

Yes, based on an analysis of the evidence we conclude that lemons are a conditional host, with the exception of green to light yellow lemons. There is uncertainty about the shade of light yellow at which

lemons change from being non-susceptible to becoming susceptible, but an overripe or over-mature condition leads to susceptibility, especially in the presence of high Medfly densities.

Are differences in the host status of varieties significant?
This is not known as it has not yet been adequately tested. However, different varieties are harvested at different maturities, and maturity appears to be more important than varietal differences. Additionally, it is thought that color correlates with maturity in those varieties covered in the evidence studied. Harvest season, which can be a varietal characteristic, is also important as it relates to fruit fly populations present before and during harvest. For example, harvest occurs in the Fall in Argentina. At that time, the normally low populations drop and exhibit minimal activity as temperatures drop. In contrast, some lemons are harvested in the late Summer in Spain when normally high populations become very high and exhibit an elevated level of activity.

Is more research needed to answer these questions and, if so, what protocols should be followed and where should it be done?
Yes, a protocol has been designed by USDA Agricultural Research Service (ARS) and APHIS to be given to potential exporters to be executed in cooperating institutions in interested countries.

Table 1. Analysis of evidence for lemon (*Citrus limon*) as a host of Mediterranean fruit fly (*Ceratitis capitata*)

SOURCE	METHOD	HOST STATUS OF LEMON CLAIMED BY AUTHOR	CONDITION REQUIRED FOR MEDFLY HOST STATUS OF LEMON
Willink 2007 1-4 unpublished + Salvatore et al 2004*	After Cowley 1992; year-round trapping, NW Argentina (AR) multiple commercial groves, one pack house, rearing/cutting fruits from trees and ground, probably included green, 5 varieties, yellow and gold, pack house for export and culls, forced field and lab, lab reared and natural flies, no positive controls, 3 yrs, >300,000 fruit	"No immature stages found; fruit flies did not develop"	Flies present or absent, NW AR, year-round, Eureka, Lisbon, Limoneira 8 A, Fino, and Genoa varieties, green?, yellow or gold, ground or tree, damage unknown, at pack house export quality or cull, forced field or lab, fresh harvested fruit with all rind chemicals present.
Jang eurekapp2 no date [2000] + lemonpp1 no date [2000] + Fruit maturity ARS methyl bromide alt 2001	California, Eureka variety, dark green through bronze, lab flies, harvested fruit in lab, analysis of peel pressure and chemicals	Fruits more mature than silver stage were "infested" by Medfly. There was oviposition in light green fruit but no recovery. More Medfly were recovered as the fruit ripened. Penetration pressure of peel is reduced as fruit ripens. Peel oil data is inconclusive between maturity stages [probably if harvested same date]	Eureka, dark green through light green had eggs but no development, silver or more mature infested, then the more mature and the less peel pressure, then the more infested
Spitler et al 1984	Lab cage w/ lab reared flies in Hawaii, 1-3 day exposure, rearing/cutting, commercial, green and yellow, California, Eureka and Lisbon, 1-2 days off tree, 7 months, 13 trials, positive peach controls	"would not support development", pupae recovered in "ripest" = survival, 5 pupae/516,000 eggs > probit 9 efficacy, probability of infestation "extremely low"	Commercial Eureka and Lisbon, green to yellow, off tree 1-2 days, infest in cage w/ lab flies
Sproul 1976	Western Australia, lab flies, cage studies on harvested commercial Eureka, green export grade to yellow overripe	In green export grade eggs hatched but firsts died, pupae only from yellow overripe, pupae development "most unlikely" on "unripe"	Green export grade Eureka
APHIS Trip report Spain Sept 2006 + 8 11 06 EAN + dead Medfly 2	Spain, Verna and Fino, commercial, yellow through green, on ground, on tree, Spain, Verna pack house export and culls, natural infestation checked by cutting, also Verna imports cut at POE	Infestation of live thirds in Verna at POE with sample of 30/container, infestation of dead thirds in one fallen Verna per 400, none in Fino	Green fruit not infested, yellow fruit could be infested under drought stress condition, poor shape and size quality, overmature, low turgor, high fly population, late season

Carey 1984	Greece, Lab flies, washed fruit in cages, females oviposit, cut and rear fruit, also firsts placed on fruit piece	Whole fruit, oviposition occurred, pupae recorded on pieces of fruit 32% emergence occurred, noted [generally] young larvae could not penetrate rind of "citrus" and died as larvae, develop on pulp	Larvae reaching pulp, no variety noted. Greece.
Saafan et al 2005	Egypt, 2 years, trap monitoring, rearing fallen fruits in lab, determined % natural infestation, fruits probably dark yellow	Yr1 3 fallen fruits=5 adults-Yr2 9 fallen fruits=3 adults, Flies per trap per day (FTD) < 0.7 both years, no zeros	Flies can be present, no variety noted, fruit fallen probably dark yellow, infestation rate high, Egypt
Bodenheimer 1951	Palestine, other, review	Observation of oviposition punctures, reports Marteli 1914 got no infestation in cage studies, and no infestations have been observed in Palestine, from green to overripe he observed # oil glands decreases and flavedo and albedo thickness decreases and toughness of albedo decreases	Regardless of Savastrano 1914, high acidity in pulp is not a resistance factor; in citrus, gum, oil, and hard rind resistance factors to firsts, persistent oviposition into the same hole may breech the rind to the pulp, "practically immune" in Palestine
Hanna 1948	Egypt, commercial, no variety or maturity, resistance study	Common acid lemon "immune' based on chemical and physical factors	Egg survival--Increased resistance with low pectin, low water in rind, low humidity of air, and extended length of development of eggs
Back & Pemberton 1915	Hawaii, no variety, commercial or not, smooth and rough	Natural infestation "ripe" 0 larvae got into pulp in 50 fruits, no punctures in green to turning yellow, larvae develop to "mature" when transferred to green or ripe lemon, mortality of eggs is in rind, most larvae die before reaching pulp, larvae in pulp only in "overripe" fruit, no mention of pupae or adults reared out	Only overripe lemons susceptible to mature larvae making it to pulp due to successive attacks by females in same puncture overcoming oil glands
Back & Pemberton 1918	Hawaii, no variety and "California", fruit natural infestation from tree or ground, held and reared out to mature larvae, review, of	Not support Medfly unless rind damaged on tree first	Tough albedo and intact oil glands, undamaged rind, and not letting them get overripe and exposed to successive ovipositions

	35 infested fruit none had larvae in albedo or pulp after holding, Quayle 1914—15 infested fruit in "thousands" and 8 weeks in Europe, all associated with peel injury, supported by Martelli and Savastrano, no larvae in Hawaii in smooth or rough lemon unless damaged rind—1/235 commercial lemons from tree developed adults, but it was damaged, 2/161 commercial from ground, 0/434 rough from ground, 1/1 rough from ground had thirds but no adults; lab cage exposure, fresh picked and "cured"—larvae but no adults		are resistance factors
Avidov & Harpaz 1969	Israel, observation, commercial or not, no variety	"resistant" but "attacked"	Fruit yellowed on broken boughs, water deficit, and "ripening" in summer are "attacked" ; prickly pear attacked only when flies at high levels
Cirio et al 1972	Italy, survey, commercial, oviposition holes and cutting for larvae	Larvae rare in lemon late in season, less preferred host is attacked when population of fly is high	High fly population
Quayle 1929	Review, no variety, commercial and not commercial	Develops in overripe and partially decayed, condition of rind important to limiting oviposition or hindering development of firsts	Host if partially decayed, overripe, damaged rind
Quayle 1914	Sicily, report, commercial, no variety, ground and tree, also lab cage infestation tests	Page 8: 15 infested per thousands checked, larvae in ground and tree fruit but only overripe and partially decayed, none of the fruit of market quality, notes 1 larva in Hawaii good quality lemon though infestation "rare", lab cage negative punctured and unpunctured	Not stated
Papers below were reviewed but provided less specific information used in making			

conclusions than the above papers			
Zucoloto 1993	Brazil, supermarket frozen and washed fruit, lab flies, firsts placed on pieces of fruit, cage females laid eggs on pieces of fruit, cage females laid eggs in whole fruit, firsts given choice of positive control or test pieces of fruit, caged females given choice of whole positive control or test	"adults did not emerge" from firsts placed on pieces of fruit, 0.0 ovipositions on pieces, "no emergence" from cage females laying eggs in whole fruit,	No oviposition observed. "Low nutritive value" given as reason no emergence. Difference in susceptibility of the same fruit species grown under different environmental factors and differences in "types of selection" by different populations of flies mentioned as factors. Damage of peel noted as oviposition factor. No variety noted. Brazil.
Radonjic 2006	Montenegro, Lunario and Meyer (may not be *Citrus limon*) varieties, adult monitoring, non-standard lures, commercial and mixed orchards, 2 years, "semi-ripe" fruit "suitable" for infestation collected, "inspected" for larvae	FTD > 1. "symptoms" of infestation in lunario in some localities late Sept-Nov. Adults present, widespread.	Adults can be present, larvae can be found
Laborda et al 1990	Spain, lab flies, diet control, injection of eggs into pulp, no variety, no maturity, reared out	"High larval mortality", slow development, 3.3% of 24,000 eggs injected into pulp of 120 fruits produced adults	High larval mortality attributed to high acidity and low sugar of pulp
Krainacker et al 1987	United States, lab flies, source of host not known, no varieties, "peak of ripeness", eggs placed on pulp and adults reared	12% survived to preadult, lowest survival host, adults fecund	Eggs placed on pulp, Medfly female a generalist that tries new hosts
Papaj et al 1989	Greece, Punctured and unpunctured oranges, not lemons, observed for natural oviposition	not studied	Punctured more attractive to females, oviposition than unpunctured
Katsoyanos et al 2002	Greece, damaged flavedo citrus, wild flies, observation of attraction	Not studied	Females no difference damaged vs. undamaged flavedo
Verain Palacios 2005	Argentina, commercial, citrus pests	Not studied	Infests citrus
Garcia-Mari 2006	Spain, lemons	Infests "early ripening fruits"	Not stated
Fimiani 1989	Turkey, commercial lemons	Infestations not important report from EPPO,	Not stated
Ovruski 2003	NW Argentina, 30 sites, 6 years, 2 month peak fruiting period, reared out natural field infestation, lemon unknown cultivar, fallen "ripe" or "ripe" fruit on tree, fruit rinsed Nabenzoate	426 fruit, 6 sites not found infested, 4 other types citrus found infested	Not stated

	before holding, reared F2s		
Mavrikakis et al 2000	Crete, adults monitored for 3 years, oranges "with distinct oviposition stings" collected bi-weekly to rear out and cut, no varieties of lemon mentioned	Apparently not found, but not mentioned other than "host", all stages present and oviposition occurs year-round	Not stated
Woods et al 2005	Australia, Natural field infestation reared out adults, 4 years, no variety, tree and ground, green or yellow, not commercial	No Medfly reared, 21 fruit	Could use lemon if Medfly populations high level and other hosts not plentiful
White & Elson-Harris 1994	World review	Recorded hosts of Mediterranean area Fimiani 1989; recorded host of Harris 1989 and others in Hawaii, Spitler 1984 recorded that some varieties not infested California, only attacked when damaged PNKTO 26	Not stated
Eskafi 1988	Guatemala, 2 years, not commercial, Eureka and others, green to fully ripe, adults reared from natural field infestation	1% infestation, 0.73 pupae/kg Eureka, 1 pupa/ 310 kg lemons, green or medium-ripe, 0.7-1.0 pupae/kg in green Eureka	Green lemons low infestation
Thomas et al 2005	World review	*Citrus* x *limonia* lemon and *Citrus limon* "heavily or generally infested" except for smooth skinned sour Eureka, Lisbon, and Villafranca	Three cultivars of smooth skinned sour lemon not infested; *Citrus limonia* not a lemon
Thomas et al 2001	World review	Life cycle extended in lemon	Not stated
Siebert & Pradhan 1991	California, Review, commercial, economic	Lemons of California not considered host; Other lemons considered host; Lemon a regulated article except for smooth skinned lemon of commerce that is cleaned and waxed	smooth skinned lemon of commerce that is cleaned and waxed
Quilici et al 2001	Seychelles	No emergence	Punctured, garden and grove
Australia 2005	Australia regulated host list	Host	Not stated
Malavasi et al 1980	Brazil, *Citrus limonia* Os., survey reared out natural infestation	Not host; *Citrus limonia* [not = *Citrus limon*]	Not commercial
Liquido et al 1991	World evidence review natural and lab infestation	Some host some non-host	Not concluded
Liquido et al 1990	Hawaii, fruit collection, natural field infestation, adults reared out, no variety, 1949-1985	18/800 2.2% of fruits produced adults	Not stated

Hancock et al 2000	Australia, host list based on 2 previous workers cited	Host	Not stated
Farias & Nakagawa 1970	Hawaii, "host" trees used as places to hang traps	"*Citrus limon*, lime" name confusion	Not stated
De Meyer et al 2002	Africa, check list, based on "reared material" checked by authors	Host, Kenya	Not stated
Copeland et al 2002	Kenya, natural infestation, reared out	Host, Kenya, list from previous worker study, not sampled in this study	Not concluded
CABI 2006	World, host list	Minor host	Not stated
Brown no date	Florida, review	Larvae take longer to "reach maturity" in lemon	*Citrus limon* and *Citrus* x *limonia*, except for Eureka, Lisbon, and sour lemon
Australia 1989	Australia, Host list	Host requiring treatment A list, no exceptions or required conditions	*Citrus limon* x *C. chinense* and *Citrus meyeri* under all conditions

*The following publication of this material, with additions, appeared on the internet in December, 2007, too late for review, but the conclusions remain the same with the additional conclusion that Medfly is called a "Conditional non-host" [after the APPPC (2005) protocol] because adults were reared only from artificially damaged fruit exposed to females in a cage test. The following were Chapters in Willink, E.; Gastaminza, G., Stein, B.,Augier L. (Eds.), Fruit flies and its [sic] quarantine relevance in the citriculture of Northwestern Argentina; eleven years of research 1996-2007. Estación Experimental Agroindustrial Obispo Colombres, Tucumán, Argentina, 143 pp. Online at www.eeaoc.org.ar. :

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