Host Susceptibility of Blueberry (*Vaccinium* spp.) to Mediterranean Fruit Fly [*Ceratitis capitata* (Wiedemann)] and South American Fruit Fly [*Anastrepha fraterculis* (Wiedemann)]

Summary

Currently available information shows that, under certain conditions, blueberry may be a host of Ceratitus capitata (Medfly) and Anastrepha fraturculus (South American fruit fly). Host expansion to olives in Peru for the Medfly and positive rearing experiments from blueberry in Argentina represents evidence that Medfly and A. fraterculus sometimes infest new hosts. More research is required to identify whether certain species of Vaccinium are more resistant to Medfly and A. fraterculus and could be considered non-hosts. The absence of any such data at this time argues for regulating all species of blueberries in the same manner as regards their host status for Medfly and A. fraterculus. Considering the high invasive potential and apparent ecological plasticity of Medfly and due to the fact that A. fraterculus is a species complex and can possibly infest different hosts in different areas, tests confirming the absence of larvae should be required for fruit movement from infested areas to non-infested areas with potential hosts.

I. Introduction

APHIS does not currently regulate the movement of blueberries to and from quarantined areas during an outbreak of Mediterranean fruit fly (Medfly) because blueberries have not previously been considered a Medfly host. Past risk assessments did not conclude that blueberry or other species of *Vaccinium* were hosts of the Medfly based on reviews of somewhat inconclusive scientific literature. The same is true regarding the South American fruit fly, *Anastrepha fraterculus*. Recent information from Argentina indicates that blueberries are a host for Medfly and *A. fraterculus* under specific conditions.

Host status is a key criterion in deciding whether regulatory measures are required to mitigate the pest risk associated with Medfly in any commodity, including blueberries. Understanding the host status of blueberry is critical to identifying regulated articles for the purpose of implementing 7 CFR 301.78, the current Mediterranean fruit fly quarantine. The quarantine is designed to prevent the spread of Medfly during an outbreak in the United States and thereby protect the $2 billion fruit and vegetable industry in the United States from the spread of the pest.

This study summarizes the results of a review of scientific literature and current research on the host susceptibility of all species of blueberries (*Vaccinium* spp.) for Medfly and *Anastrepha fraterculus* and provides a recommendation on the status of blueberry as a

host. Consideration is given as to whether there is a technical justification for listing the entire genus or providing separate listings for individual species within the genus.

II. Methodology

Species of *Vaccinium* belong to the family Ericaceae and include blueberry, cranberry, lingonberry, highbush and lowbush blueberries, rabbiteye blueberries (NCGR, 2006), ohelo berry (Back and Pemberton, 1918) and several others. Records were collected from the literature, unpublished scientific results, or other types of reports addressing the relationship between *C. capitata, A. fraterculus* and any of the *Vaccinium* species to determine their potential host status. Records that list *Vaccinium* spp. as a host but do not include any field or laboratory research in support of this statement (Liquido *et al.,* 1991, 1998 and references therein) or those that name *Vaccinium* spp. to be a host of "unknown significance" (Thomas *et al*., 2001) were considered inconclusive and were therefore excluded from the analysis.

III. Results

Most published sources do not identify *Vaccinium* species as a suitable host for *C. capitata* (e.g. CABI, 2005; EPPO/CABI, 1997; EPPO, 2005). Others list *Vaccinium* spp. as a host but do not include any field or laboratory research in support of this statement (Liquido *et al.,* 1991, 1998; Thomas *et al*., 2001). There were no records [except Vaccaro and Bouvet (2006) discussed herein] that listed any of the *Vaccinium* species as hosts for *A. fraterculus*. As a result, this study is largely based on recent information from Argentina where specific field and laboratory studies were conducted in order to identify suitability of *Vaccinium* spp. as a host.

Two studies, conducted independently in different regions of Argentina, presented conflicting results regarding host suitability of blueberries for Medfly (Aceñolaza *et al*., 2003; Vaccaro and Bouvet, 2006). Aceñolaza *et al*. (2003) conducted their research in Famaillá (Tucumán Province) from 2001 to 2003. This region is located in the north-western, mountainous area of Argentina (Appendix 2). The studies resulted in no captures of flies in Jackson and McPhail traps in plantations of different varieties of blueberries. In addition, neither eggs nor larval infestations were found in the samples of mature blueberry fruit. The researchers did find 20 adults of *C. capitata* in the traps placed in the production area of peach (*Prunus persica*), an alternative host. No numerical data are available regarding numbers of either traps or fruit samples taken for rearing. Although the authors considered their research preliminary, they concluded that it could be a basis for determining the non-host status of blueberry. The results were presented in 2003 by Instituto Nacional de Technología Agropecuaria (INTA) as a poster for an unspecified professional congress. The level of uncertainty associated with the results from this study appears to be high.

Vaccaro and Bouvet (2006) started research monitoring *C. capitata* and *A. fraterculus* in blueberries in 2003 in different production areas: Tucumán, Buenos Aires, Corrientes and Entre Ríos, where the Medfly populations were registered (Appendix 2). Trapping was

conducted in two 20 hectare plantations, north and south of Concordia, Entre Ríos, from early September of 2005 to mid-January of 2006. In each plantation, five Jackson Traps with Trimedlure bait and five McPhail Traps with Torula yeast bait were used. Traps were checked weekly. The traps were placed within the plots and in their perimeter, as well as on citrus trees found in the plantation. The authors recorded an increase in the Medfly captures from mid-November to early January in the northern orchard and a peak of the population in the southern orchard at the end of November. Presence in the blueberry plots of the alternative host, citrus, in unidentified phenological stage, adds uncertainty to the report on trapping results.

Fruit from 14 cultivars of *Vaccinium corymbosum* and two cultivars of *V. ashei* were sampled in seven plantations in Concordia (Entre Ríos) and in one plantation in Curuzú Cuatía (Corrientes) for the laboratory rearing of Medfly. Fruit from all the plantations checked were attacked by one or both species with different infestation degree.

A total of 111,850 grams (246 lbs) of blueberry fruit samples were collected from which 522 flies of *C. capitata* were obtained. The number of flies found depended on the cultivar and varied from 1 to 283. Some cultivars showed no infestation (Appendix 1). The level of infestation increased with population density and reached its peak in November and December with the blueberry export season generally concluding at the end of November (Vaccaro and Bouvet, 2006). In general, higher infestation was observed in older plantations.

From this collection, 230 *Anastrepha fraterculus* flies were obtained. Not all cultivars were equally susceptible to the fly infestations, with levels ranging from 0 to 185. This is the first record, to our knowledge, where species of *Vaccinium* were recorded as a host for *A. fraterculus*.

Vaccaro and Bouvet presented their studies as a poster at the 7th International Symposium on Fruit Flies of Economic Importance, held in Salvador, Bahia, Brazil in September, 2006. These results of fruit fly rearing appear very convincing.

It is also important to mention an OPIS report from Argentina dated December 9, 2005 (OPIS, 2005). The report describes the visit of APHIS-IS representatives to a blueberry farm in Curuzú Cuatía, (Corrientes) [note: same department where some of Vaccaro and Bouvet's (2006) studies were conducted]. The farm is located in an isolated area of the province. During the visit, the APHIS team collected blueberry fruit from the ground in the field and confirmed that several contained fruit fly larvae. Several fruit fly larvae were also discovered in the packing house where culled fruit from the packing line was inspected.

In the previous week, an entomologist from Instituto Nacional de Tecnología Agropecuaria (INTA, the Argentine equivalent of the USDA Agriculture Research Service) also collected several fruit fly larvae at the same farm and identified them as *C. capitata*. The infested blueberry field was located in a remote area with the nearest other Medfly host material in a citrus grove one kilometer away. Photographs of blueberry fruit infested with fruit fly larvae accompany the report (OPIS, 2005).

APHIS and SENASA also visited another blueberry farm in Corrientes, near the town of Juan Pujol, 80 km south of the first farm. No fly infestations were found in the fruit collected from the ground, from the bushes, or from culled fruit in the packing house.

IV. Discussion

Conflicting evidence and the absence of more extensive observations and research increases the degree of uncertainty. The evidence strongly suggests that Medfly and *Anastrepha fraterculus* are capable of infesting *Vaccinium* species under particular conditions that can sustain the pest population when a preferred host is not available.

Results of the trapping experiments seem to be the most uncertain in the evidence (Aceñolaza *et al*., 2003; Vaccaro and Bouvet, 2006). This is primarily due to limitations of the experimental design. The results of fly rearing from infested commercial blueberry fruit (Vaccaro and Bouvet, 2006), are the most convincing evidence of the host status of *Vaccinium* spp. but do not provide any insight into the basis for the difference in host status in different areas.

Medfly is a highly polyphagous insect with a host range of more than 300 plant species, primarily temperate and subtropical fruits (Liquido *et al.,* 1991). The species is highly invasive and spread from its native range in the Mediterranean region and North Africa (Mau and Kessing, 1992) to North America, South America, and Australia (Thomas *et al.* 2001) where it expanded its host range. Host preferences vary in different regions (Mau and Kessing, 1992). Both Corrientes and Entre Ríos are part of the Mesopotamic region and are situated in the almost entirely flat, low land and surrounded by several rivers. The climate in this region is different from the climate in geographically distant, mountainous area of Tucumán (Appendix 2), possibly causing differences in chemical composition among the same host species that might affect their suitability as hosts for Medfly.

The ease with which Medfly infests new hosts could also be explained by studies of Prokopy *et al*. (1984) who found existence of "significant inter-populational differences in propensity to … (accept) various types of fruit for oviposition among *Ceratitis capitata* females from two wild sources and one laboratory source". The researchers state that "at least a portion of the inter-populational variation had a genetic basis" (Prokopy et al., 1984).

An example of infesting a different host in a new geographic area is infestations of olives by Medfly in Peru (Avocado PRA Peer Review Report, 2006). Olives are generally not considered to be hosts of Medfly. The only record we are able to find was reported from Kenya where Copeland *et al*. (2002) were able to obtain Medflies from wild olives, *Olea woodiana* Knobl. (5 flies/758 fruits) and European olive, *Olea europaea* L. ssp *africana* (Mill.) P. Green (1 fly/2306 fruits). Although neither of the two *Olea* species was heavily infested, "… the records of *O. woodiana* demonstrate that they are acceptable hosts" (Copeland *et al*., 2002). The authors suggest that, in the case of *O. europaea* ssp. *africana*, its host status needs to be further confirmed because *Olea europaea* ssp.

europaea L. was previously reported to be unsuitable for oviposition. The difference in oviposition preferences might be due to the "remarkable difference" (Copeland *et al*., 2002) in fruits between these two species of olives.

In Peru, the region of Southern Lima and Ica are the only locations throughout the range of *C. capitata* where this species infests olives as a host plant. Reports have suggested a high infestation rate. This could indicate that populations of Medfly in these isolated oases along the Pacific have broader host range than is typical for the species (Avocado PRA Peer Review Report, 2006). The same reviewer also suggests that "Peruvian *C. capitata* may also have some taxonomic or unique ecological characteristics".

The genetic variation in Medfly populations may not necessarily be high. Studies by Callejas and Ochando (2004) of variability in populations of *C. capitata* from the Iberian Peninsula (eight wild samples and one laboratory strain) found that only four loci of the 15 loci studied were clearly polymorphic. There were no significant differences in populations collected from different hosts (Callejas and Ochando, 2004). The authors speculate that this low variation is the adaptation of the Medfly to different fruit hosts. The population infests different hosts depending on their availability. Consecutive generations, depending on time of the year, must be adapted to different hosts. This would tend to restrict variability since natural selection would favor more versatile "generalist" type alleles that could serve for different hosts (Callejas and Ochando, 2004).

In the absence of significant genetic differences in Medfly populations from different hosts, attractiveness to alternative hosts may perhaps be explained by a similarity between the hosts in their olfactory composition. Prokopy *et al*. (1998) found that protein-fed females of Medfly were responding to volatiles of 2-heptanone as if it were an oviposition site stimulus. This compound was initially found to be attractive to Medfly by Warthen *et al*., (1997) who isolated it from coffee. Previously, 2-heptanone was identified from blueberries (Lugemwa *et al*., 1989).

The above olfactory similarity between coffee and blueberry (host vs. host of unknown significance) could be an important explanation for the fly's choosing blueberry for oviposition. In earlier studies, Prokopy *et al*. (1996) concluded that "odor of natural food of Medflies could lure flies to plants whose fruit emit little or no attractive odor and are not permanent hosts but which are nonetheless susceptible to egg-laying and larval development, resulting in temporary expansion of host range".

Anastrepha fraterculus is known to be a species complex that has not yet been studied in sufficient detail to permit a clear separation of the included species (White and Elson-Harris, 1992): thus, in Venezuela, Andean and lowland populations are distinct species, and populations from southern and north-eastern Brazil also have marked genetic differences. Also, "there is evidence that the Mexican morphotype differs significantly from South American morphotypes" (Aluja *et al*., 2004 and references therein).

Anastrepha fraterculus is variable in its pest status in different regions, and isozyme and karyotype studies suggest that what has been considered *fraterculus* consists of several

closely similar "sibling species" (Foote *et al*., 1993 and references therein). There are some genetic differences between *A. fraterculus* collected from *Psidium guajava* L. in the Buenos Aires (central-eastern region) and Tucumán (northwestern region) Provinces (Sonvico *et al.* 1996), however Alberti *et al*. (2002) concluded that Argentine populations of the complex are conspecific.

In Argentina, *A. fraterculus* is mainly restricted to the northern region between 22° and 31°S latitude where it breeds in native and wild exotic plant species (Ovruski *et al.* 2003), whereas *C. capitata* occurs from the northern region to as far south as 40°S latitude in Patagonia (southern region), mainly in the Río Negro Valley, commonly infesting commercial exotic fruits (Sanchez *et al.* 2001).

Ovruski *et al*. (2003) and Orono *et al*. (2005) reported that, in northwestern Argentina, *A. fraterculus* is much more abundant in native, wild fruit than *C. capitata*. These authors showed that *A. fraterculus* appears to prefer areas with patches of wild vegetation, whereas *C. capitata* seems to adapt well to highly perturbed environments where exotic plants are more common. A similar situation has been recorded in several regions of Brazil (Malavasi & Morgante 1981; Malavasi et al. 2000).

Also relevant in this analysis are certain public comment submissions from Argentina in response to the Proposed Rule published by APHIS in the Federal Register in 2005. Concerns were voiced by Argentina regarding the situation with blueberry field infestations by Medfly and importation into the United States of fruit without treatment (Regulations.gov, 2006 query). It seemed unusual for growers from the exporting country to comment in such a manner.

For example, Jorge Pazos, the President of CAPAB (Argentina Blueberry Grower Chamber), an organization that represents almost 80% of the blueberry growers in Argentina, suggests conducting more studies "…to determine the behavior of the Medfly regarding to blueberries…" in the new areas of production. In those areas, the Medfly is a serious problem in citrus and the density of fly populations is high (Public Submissions to Document ID APHIS-2005-0027-0019/0020, 2005).

Felipe Rodríguez, the General Manager of Tecnovital (the largest blueberry export company in Argentina) states that they are in agreement with the new rule but would like to know the conditions of blueberry export "… since although we are sure that the blue (sic) are not a Medfly host our fruit proceeds from a country that is not free from this insect" (Public Submissions to Document ID APHIS-2005-0027-0017/0018, 2005). It seems unusual for exporters to be concerned with the Medfly in the product which is believed to be a non-host since Medfly is unlikely to be a hitchhiker.

One anonymous commenter described that in December of 2004 "… some fruit fly-bitten blueberries" (mainly O'Neal variety) were detected. This fact was confirmed by Ing. Norma Vaccaro of the INTA Experimental Station at Concordia. Furthermore, 200 adult *Capitata ceratitis* (sic) flies were found in 2 kg of discarded blueberries from a Curuzu Cuatía (Corrientes) grower …" (Public Submissions to Document ID APHIS-2005-0027-

0025, 2005). The commenter further states that despite the efforts to create a cooperative program for further studies in different areas of Argentina (with participation of SENASA), in reality, there was no consistent trapping program or analysis of samples. The commenter expressed the strong belief that identifying blueberry as non-host is inconsistent. Also, according to this commenter, Patagonia should not be declared a Medfly free area. Although anonymous, this statement could be considered credible enough since it is consistent with other reports: 1) OPIS report of December 2005 regarding findings of infested blueberries on the farm in Curuzú Cuatía, (Corrientes); 2) Dr. Vaccaro's published research (Vaccaro and Bouvet, 2006) indicating the highest infestation rate in the blueberry of variety O'Neal. It appears that the infestation in Corrientes is ongoing since 2004 and was not a single season's abnormality.

V. Conclusion

The weight of evidence suggests that blueberry is a host of Medfly and *Anastrepha fraterculus* under specific conditions. The host expansion to olives in Peru and rearing experiments from blueberry in Argentina, despite certain limitations, are evidence that Medfly can infest unusual hosts such as blueberry, under certain conditions. *Anastrepha fraterculus*, representing a species complex, is known to select different hosts depending on the areas of its distribution. There is no currently sufficient evidence to distinguish host status across different species of the genus *Vaccinium*. More research is required to identify possible differences in resistance to Medfly and *A. fraterculus* by species of *Vaccinium*. Based on the findings of Vaccaro and Bouvet (2006), not all varieties of *Vaccinium corymbosum* appear to be equally susceptible to attacks by both fruit flies. Therefore, differences in susceptibility to these flies may also need to be studied for resistant varieties within species.

Future evidence identifying resistant varieties, and the mechanism for their resistance, could be used to support reducing regulatory restrictions based on fruit fly-resistant status. In the absence of such information, the high invasive potential and ecological plasticity of *C. capitata* and *A. fraterculus* argues for considering all *Vaccinium* species and all blueberry sub-species and varieties to be a host and require tests confirming the absence of larvae in fruits for export from each area where blueberries are moved from infested to un-infested areas with suitable hosts.

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APPENDIX 1. Infestation of blueberry fruit (Vaccaro and Bouvet, 2006).

Table 1. Fruit sampled and fruit flies collected

Scientific name	Cultivar	Fruit collected	Fruit flies collected	
			C. c	A. f
Vaccinium corymbosum (Highbush)	Bluecrop	16060	0	3
	Emerald	1800	0	0
	Georgiagem	600	1	0
	Gulfcoast	900	4	6
	Jewel	1660	0	0
	Misty	11000	0	0
	O' Neal	32800	203	21
	Rexmills	12600	3	14
	Santa Fe	1360	0	0
	Sharpblue	1660	28	0
	Southmoon	900	0	0
	Star	600	0	0
	mix1	27960	283	185
	mix2	1600	0	0
Vaccinium ashei (Rabbiteye)	Premier	460	0	0
	Climax	460	0	2
Total		111860	622	230

APPENDIX 2. Map of Argentina.