

Fruit Fly (Diptera: Tephritidae) Host Status Determination: Critical Conceptual, Methodological, and Regulatory Considerations*

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Key Words

host plant, oviposition behavior, host range evolution, quarantine pest, risk analysis, systems approach

Abstract

Although fruit fly host status determination/designation lies at the heart of strategic decisions on national and international trade of fruit and vegetables, all attempts thus far to define host plant status have been contentious and as a result long-standing disputes between commercial partners throughout the world have lingered over decades. Part of the problem is that too little effort has been devoted to understanding the underlying mechanisms involved in host plant use by fruit flies and that instead economic and political interests usually prevail. Here we review the most important evolutionary, biological, ecological, physiological, and behavioral aspects that drive host use by fruit flies, and then construct a flow diagram rooted in these fundamentals that outlines a series of steps and definitions to determine if a particular fruit or vegetable (and cultivars thereof) is a natural host, or a conditional (potential, artificial) host, or a nonhost. Along the way, we incorporate risk analysis considerations and propose that the underlying complexity determining host plant utilization by fruit flies requires a flexible systems approach capable of realistically dealing with fly/host/environment/geographic variability on a case-by-case basis.

Systems approach:

integration of pre- and postharvest practices, from the production of a commodity to its distribution and commercialization, that cumulatively meet predetermined requirements for quarantine security

Natural host: fruit or vegetable unequivocally found infested under totally natural field conditions (i.e., nothing is manipulated)

INTRODUCTION

Fruit flies (Diptera: Tephritidae) are among the most important pests worldwide because of their direct economic impact (i.e., female oviposition and larval feeding render fruit/vegetables unmarketable) and the strict quarantine restrictions imposed by many countries to curtail their entry (1, 31, 64). A critical component when trying to assess the risk of introductions into a country or an entire region is the status of a given fruit/vegetable species (and cultivars thereof) as a host of a particular fruit fly species (3, 82). Given the underlying biological complexity/conditionality of fruit fly host use and the economic and political impact of the issue of host plant status determination/designation at the international level, there is an urgent need for a solid conceptual and methodological framework that can aid scientists, regulatory officials, policy makers, politicians, and stakeholders throughout the world in solving current and future disputes over the status of particular plants as fruit fly hosts.

Here we analyze the causes of confusion and complexity involving fruit fly host determination and ultimately agricultural risk, including, among others, (a) the evolution of fruit fly/host plant relationships and the behavioral mechanisms related to host finding and utilization; (b) conditional infestation based on the physiological (motivational) state of the fly; and (c) conditional infestation based on the state of the host. We propose that the underlying complexity/conditionality that determines host ranges requires a flexible systems approach capable of realistically dealing with fly/host/environment/geographic variability on a case-by-case basis. We construct a flow diagram (**Figure 1**) that proposes a series of steps and definitions to determine if a particular fruit or vegetable (and cultivars thereof) is a natural host, or alternatively (non-natural host), if it should be considered a conditional (potential, artificial) host or a non-host. We finish by outlining promising areas of research that could help solidify a stream-

lined system designed to reach science-based decisions on fruit fly host designations.

EVOLUTIONARY CONSIDERATIONS OF HOST USE BY TEPHRITID FLIES

Overview of Broad Patterns Involved

The evolutionary pathway leading to specialized herbivory is a complex one and the factors shaping host plant use are highly diverse, with patterns spanning from extreme conservatism (175, 182, and references therein) to extreme plasticity (57, 58). Tephritid conservatism is exemplified by the large subfamily Tephritinae, which is almost exclusively associated with plants within the Asteraceae (52). But there is also evidence for rapid host range evolution, with related insects attacking a wide range of unrelated plants (7, 154).

In broad terms, two principal driving forces guide host range evolution: phytochemical coevolution between plants and herbivores (47) and selective pressure by generalist natural enemies (19, 22, 87, 158). Which selective forces played a more important role in the case of tephritid flies is open to debate. Ancestral tephritids most probably evolved from a saprophagous to a phytophagous lifestyle (43). Currently, most fruit flies specialize in flowers and fruit structures that frequently lack the plants' characteristic secondary chemistry (112). Furthermore, it has been argued that domesticated fruit were selected on the basis of their low toxicity to vertebrates (84) and that artificial selection for increased human palatability also lowered the levels of chemical defenses in commercially grown fruit (80). Additionally, Fitt (58) pointed out that as fruit ripen, defensive compounds disappear. Conversely, some fruit fly species feed on seed tissue that may be more toxic than the pulp, and in the case of pulp, there is evidence that some fruit remain highly toxic to larvae (see below). For example, in the case of flies within *Anastrepha*, primitive species such as *A. cordata*, *A. hamata*,

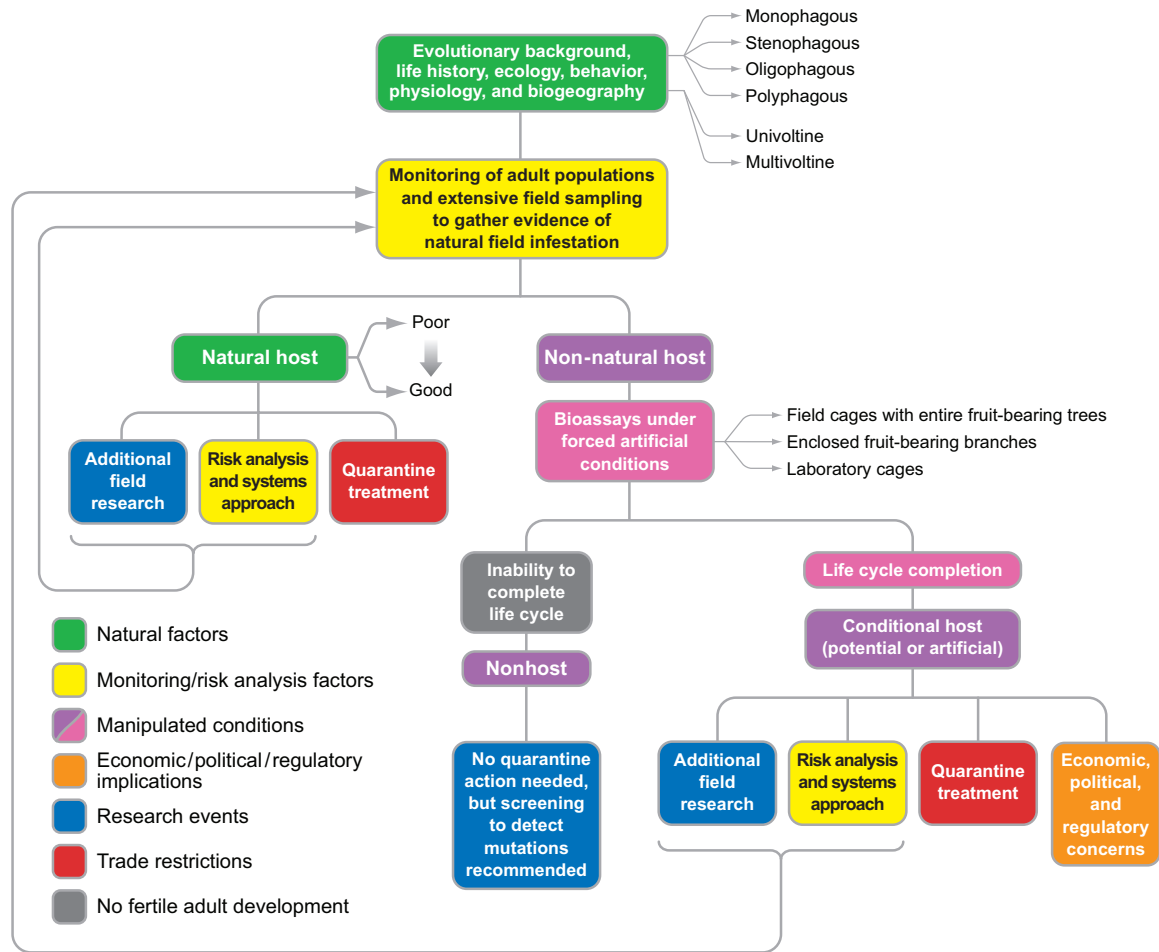


Figure 1

Flow chart indicating proposed steps for fruit fly host status determination/designation. The entire approach is rooted in the fundamentals of the system, i.e., the evolutionary background of host plant associations, life-history parameters, ecology, physiology, and most importantly behavior. When quarantine systems are based on a systems approach, the entire system should be continuously evaluated by resampling and reanalyzing the components to improve security.

A. crebra, and their closely related relatives (flies in the sister group *Toxotrypana*) specialize on seeds or associated tissue of and attack latex-producing plants (e.g., Apocynaceae, Asclepiadaceae, and Sapotaceae). In contrast, some derived species (e.g., *fraterculus* group) feed almost exclusively on fruit pulp and are highly polyphagous (7, 9). Species such as *A. ludens* (also within the highly derived *fraterculus* group) have retained the ability to feed on both types of substrates (9).

The other argument, that host ranges evolve because of the selective advantages of moving to hosts that provide shelter from natural enemies, has not been formally tested, although Feder (53) provided evidence that, at least in the case of the apple maggot (*Rhagoletis pomonella*), a shift from native hawthorns to introduced apples did allow the frugivore to gain enemy-free space.

According to Zwölfer (182) fruit flies arose in the middle Tertiary (at least by the early

Diet breadth:

determined by number of species of plants included in an insect's diet

Conditional host: a host plant unequivocally not found infested in the field but that can be infested under manipulated conditions (also called potential or artificial host).

Miocene), which means that tephritid fly-plant associations began at least 20–25 mya. For example, the highly publicized rapid sympatric host shift of *R. pomonella* [it shifted from its ancestral host *Crataegus* spp. to introduced apples (*Malus domestica*) in northeastern United States in the late 1800s] was made possible by chromosomal inversions affecting key diapause traits occurring in Mexico approximately 1.75 mya and that subsequently introgressed into the United States (54). Inversion polymorphism that generated variation in diapause schedules facilitated synchronization with the fruiting periods of the novel host (apples fruit earlier than hawthorns) (54). In addition, mutations were needed to allow females to recognize the signature odors of the new host (68). We note, however, that host shifts can also occur at a much faster pace. Observational and experimental studies on individual oviposition decisions and models such as the hierarchy-threshold model (35) have shown that individuals can shift hosts during a lifetime (70, 72, 154).

Resource Exploitation Strategies and Diet (Host) Breadth

We adopt the classification by Zwölfer (183) with respect to the resource-exploitation strategies exhibited by pestiferous tephritids: (a) opportunistic, broad-range exploiters (e.g., *Anastrepha*, *Bactrocera*, and *Ceratitis*) of pulpy fruit that are multivoltine (**Figure 1**) and very mobile, and that exhibit high reproductive potential, no diapause, and little synchronization between adult emergence and host fruiting phenology; and (b) specialized exploiters (e.g., *Rhagoletis*) of pulpy fruit that are mainly univoltine (**Figure 1**), exhibit long diapause periods in the soil, and closely synchronize their emergence with that of the fruiting phenology of their hosts.

With respect to diet breadth, tephritid flies can be placed within the following four categories: monophagous, stenophagous, oligophagous, and polyphagous (60) (**Figure 1**).

According to May & Ahmad (109) monophagous insects strictly feed on plants of only one species, oligophagous insects are restricted to a single plant family, and polyphagous insects feed on plants of many families and even orders. We find it useful to add the stenophagous category considered by Fletcher (60), which we define as species feeding on plants within one genus. Diet breadth in insects has both increased and decreased over evolutionary time (20) and therefore should be treated as a continuum (20, 79). Most species of tephritid flies, as is the case with most phytophagous insects, tend to have narrow host ranges (i.e., specialists) (87). In the case of highly polyphagous species, the issue needs to be analyzed at the species, population, and individual level because these types of insects exhibit a great degree of variability in their host use patterns (30, 40, 117). Importantly, among natural or conditional hosts, not all are equally preferred or suitable for development (poor to good host gradient noted in **Figure 1**) (8, 26, 28, 95, 100), and even among nonhosts, some are acceptable under particular conditions. The latter led Hanson (79) to conclude that the boundary between host and nonhost should be drawn in the field (i.e., at the ecological level): “Host plants are those on which the animal completes normal development in nature.”

Classifications such as those listed above may be misleading owing to local feeding adaptations. Fox & Morrow (67) indicated that “many herbivorous insects have generalized diets over the species’ entire geographical ranges but they function as specialists with restricted diets in local communities.” For example, in the case of the highly polyphagous *Ceratitis capitata*, Jones (91) discovered that aculeus morphology of several host-associated populations found in South Africa was related to physical characteristics of their hosts. Among the populations he studied, aculeus width decreased as host cuticle thickness increased, suggesting that individual populations remained associated with one host fruit species for many generations.

Constraints Limiting Diet (Host Breadth)

Although it is assumed that female oviposition decisions correspond with optimal host suitability for offspring development (18), this is not always the case (43, 143, 164). There are cases in which females lay eggs in unsuitable hosts (usually referred as mistakes, but see References 98, 110) or in which they do not lay eggs in plants (fruit) on which larvae are able to develop (57, 58). Such inconsistencies may be due to phylogenetic constraints that limit the potential behaviors and life-history strategies that can evolve (69, 128). In the case of fruit flies, we refer to the suite of receptors essential in finding hosts or recognizing surface chemicals which are constrained by the insect's anatomy, which is in turn constrained mostly by phylogeny (i.e., characters bequeathed by ancestors). Another good example of a constraint is aculeus morphology and associated sensilla (160). There are species with short and pointed (e.g., *C. capitata*), short and serrated (e.g., *A. fraterculus*), and long and pointed (e.g., *A. ludens*) aculei (91). Such characteristics may determine the type of fruit or vegetables females are able to attack (e.g., soft versus thick skinned) or the type of chemical barriers they can circumvent (23, 41). Among the economically important fruit fly genera, there is a discernible gradient when comparing aculeus length: flies within *Toxotrypana* and *Anastrepha* have the longest and flies within *Bactrocera*, *Ceratitidis*, and *Rhagoletis* have the shortest (91).

Another type of genetic constraint limiting or biasing the evolution of host associations is represented by the lack or paucity of genetic variation. According to Futuyma et al. (71), such a constraint may lead to avoidance of rather than adaptation to novel plants. Price (128) further postulated that there are "adaptive syndromes" that "are evolutionary responses to the phylogenetic constraints that minimize the limitations and maximize larval performance." Use of occupied hosts (e.g., reuse of previous oviposition punctures) or

fruit wounds is a good example of an adaptive syndrome in the case of fruit flies. For example, *C. capitata* females, which are constrained by a short aculeus, commonly use previous oviposition holes [i.e., holes left by another conspecific or the female of another species (121)] and crevices in the epicarp (epidermis) of damaged fruit caused by bird pecks (129), or they lay eggs in ripe fruit that have senesced to the point where toxic oils or allelochemicals have disappeared, allowing eggs to hatch and larvae to develop (77).

Yet another adaptive syndrome with a phylogenetic basis leading to differences in host breadth might be derived from the relative neural capacities of various tephritid lineages. According to Bernays (21), "the evolution of diet breadth in herbivorous insects is constrained by a limited neural ability to efficiently process large amounts of information in short periods of time." On the basis of this "neural-constraints hypothesis" (66, 99) and also the "loose receptor hypothesis" (162), highly polyphagous fruit flies such as *C. capitata* or *Bactrocera tryoni* would be less efficient in discriminating among a wide array of volatiles and surface chemicals when compared with a monophagous species such as *Anastrepha alveata*. Work by Fitt (57) lends support to these hypotheses. This author compared five species of *Bactrocera* that differed in host breadth [i.e., monophagous (*B. musae* and *B. cacuminata*), oligophagous (*B. cucumis*), and polyphagous (*B. tryoni* and *B. jarvisi*)] and in the extent to which they had shifted to exotic fruit. Under laboratory conditions, larvae of all species survived and developed in many cultivated fruits in which the specialist species never occur in the field. Females of the monophagous species would not oviposit in the novel fruit even in the absence of the natural host. In contrast, the highly polyphagous species would lay eggs in almost any fruit. Fitt (58) concluded that "the occurrence of these *Dacus* species [currently *Bactrocera*] in cultivated fruits is constrained more by the behavioural preferences of adult females than by larval specializations. A genetic

Phylogenetic constraints: old characters in the phylogeny of a species that can limit the sorts of behaviors that can evolve

Host quality: characteristics of a host (usually a fruit) evaluated by a female prior to the act of oviposition

change in some aspect of host recognition or acceptance would be necessary for the specialist species to regularly infest cultivated fruits though no change in larval characteristics may be needed.”

BEHAVIORAL ASPECTS RELATED TO FRUIT FLY HOST ASSOCIATIONS

The Catenary Process Involved in Host Finding and Utilization

Hassel & Southwood (81) postulated that a foraging insect perceives the environment at three hierarchical levels: habitat, patch, and food item. A discussion of critical habitat and patch characteristics influencing fly behavior can be found in References 2, 5, 6, 134, and 155. Writing on parasitic Hymenoptera, Doutt (45) divided the catenary (i.e., step by step) process of successful parasitism into host-habitat location, host finding, host acceptance, and host suitability. Vinson (173) then added a fifth component: host regulation. According to the rolling fulcrum model of Miller & Strickler (111), in this catenary process there is a complex interplay of external and internal excitatory and inhibitory inputs that lead an insect to engage or not engage in a particular behavior (e.g., oviposit).

What are the most critical factors at play during the catenary process that lead to the eventual acceptance of a host by a gravid female fruit fly? At the habitat and patch level, visual cues such as plant color, silhouette of tree against the sky (shape), size (all nonspecific to hosts), and particularly the odors emitted by a fruiting tree (mainly but not exclusively host specific) lead a foraging female to a host plant (3, 10–12, 62). Light & Jang (101) noted that there are three types of volatiles that can draw females (or males) to a particular habitat, patch, or single tree: (a) general (i.e., not tied to a particular plant species) “green leaf volatiles” such as aliphatic aldehydes and alcohols emitted by leaves and unripe fruit; (b) volatiles, mainly esters, emitted by ripen-

ing fruit; and (c) male sexual pheromones, volatiles emanating from rotting fruit, bacteria, and other food sources, and species-specific volatiles emitted by the hosts of specialized fruit fly species. After alighting on a plant, vision becomes more important, as females identify and approach individual fruit according to their spherical shape, size, and color (11, 131, 132). But importantly, if fruit are not fully ripe (a stage of development preferred by most females to oviposit), there is an interaction between chemical and visual cues that helps the female orient herself while moving from leaf to leaf or branch to branch (11). While females are flying from leaf to leaf (11) they apparently assess plant architecture, leaf chemistry, or leaf physical properties (44). Once on a fruit, females assess surface texture and chemical properties with their tarsi and decide to bore or not. If the decision is to bore and the skin is pierced, sensors at the tip of the aculeus (137) send the last series of signals, allowing the female to reject the fruit or to accept it and lay a batch of eggs. For example, sugars such as glucose and fructose (73) and protein (65) promote egg laying.

Factors that Influence Fruit Fly Oviposition Behavior

A detailed description of the highly stereotyped oviposition pattern exhibited by most pestiferous fruit fly species can be found in Reference 7. In broad terms it involves arrival on fruit, examination (head-butting), aculeus insertion, egg deposition, aculeus cleaning, and in most species, aculeus dragging (i.e., host marking) (**Supplemental Video 1**, follow the Supplemental Material link from the Annual Reviews home page at <http://www.annualreviews.org>).

Some of the most important factors that influence fruit fly oviposition behavior and that might lead a female to lay eggs into a fruit outside of its natural host range in nature or in experiments under artificial, laboratory conditions are host quality [i.e., size, color, penetrability, degree of ripeness,

presence of host-marking pheromone (HMP)] (41 and references therein), genetics (i.e., variability within and between populations) (35, 69, 70), learning (122, 130, 147), number of ovarioles (i.e., potential fecundity) (59), ovarian dynamics [e.g., egg load and concomitant oviposition drive (motivation), ability to resorb oocytes, egg versus time limitations] (4, 40, 59, 120), aculeus wear (92), age (42), social context (facilitation, competition) (46), chemical context (i.e., presence of fruit volatiles and sexual pheromones) (4), and individual variation in oviposition decisions. Of these factors, five in particular can play a critical role when conducting artificial, laboratory tests: (a) ovarian dynamics and oviposition drive (i.e., motivation), (b) learning, (c) age and the concomitant aculeus wear in females, (d) social context, and (e) genetic and rearing background (i.e., wild versus lab-reared flies) of the particular population from which flies are drawn to conduct bioassays (37).

With respect to ovarian dynamics, three life-history components are worth highlighting: (a) the relationship between the number of ovarioles (i.e., potential fecundity) and breadth of host range, (b) the ability to resorb oocytes, and (c) host use patterns with respect to egg and time limitation. There is a dynamic relationship between these factors and oviposition drive (i.e., motivation). Fitt (59) reported that highly polyphagous species within *Anastrepha*, *Bactrocera*, and *Ceratitis* had more ovarioles (higher lifetime fecundity) than more specialized monophagous and oligophagous congeners. Furthermore, specialized species are usually able to resorb oocytes and do so when deprived of their preferred hosts (59). In contrast, oocyte resorption has never been shown in highly polyphagous species such as *C. capitata*, *B. papayae*, *B. tryoni*, and *A. ludens*. It follows that in general terms specialized species are egg limited, whereas highly polyphagous species are time limited. According to Papaj (120), “egg limitation occurs when females deplete their egg supply before opportunities to oviposit are exhausted . . . Time limitation

occurs when females die or otherwise lose reproductive competence before all mature eggs have been laid.” The same author (120) goes on to say that “a high risk of egg limitation should cause females to become choosier with respect to the quality of hosts used for oviposition” and “a high risk of time limitation should, in contrast, cause females to adopt strategies that increase the rate at which hosts are found, even if such strategies reduce the quality of hosts on which eggs are laid.”

Because opportunistic generalists can utilize many species as hosts (e.g., *C. capitata*), the seasonal abundance of hosts and associated opportunities to lay eggs for these species are higher than for monophagous species (2, 57, 58). But there is a trade-off between abundance and predictability, in which the latter is higher for a specialist. As a result, whereas the life cycles of specialist species are synchronized with the fruiting phenology of their hosts (183), in highly polyphagous species that is not the case. Because highly polyphagous species cannot resorb oocytes and can in general terms be placed within the time-limited, neurally constrained categories, when females are deprived of oviposition opportunities they accumulate eggs, which in turn increases their drive (motivation) to lay eggs and lowers their preference thresholds (“electivity” sensu Singer) (152). That is, the probability of host acceptance increases with time since last oviposition (see Reference 88 for an in-depth discussion of this relevant topic). Thus, it should come as no surprise that a sexually mature *C. capitata*, *B. dorsalis*, or *A. ludens* female, with a high egg load, exposed to a fruit or vegetable not infested under natural field conditions will readily oviposit into the novel host (which would not happen with a highly specialized species such as *A. cordata* or *B. cacuminata*). Because larvae of many species can develop in plants not used in nature, it is likely that such an artificial setting can result in infested fruit. For example, as early as 1916, Back & Pemberton (15) documented artificial infestations (i.e., laboratory, not field

conditions) in bananas by the Mediterranean fruit fly and Baker et al. (16) documented infestations by *A. ludens* in bell peppers, tomatoes, walnuts, and prickly pears (also see **Supplemental Figure 1**, follow the Supplemental Material link from the Annual Reviews home page at <http://www.annualreviews.org>). As discussed below, what we are actually achieving with such procedures is determining “the absolute limits to an insect’s host range” according to van Klinken (171).

Again, the elegant work by Fitt (59) illustrates the above scenario. He showed that when females were deprived of their preferred hosts for up to 16 days prior to being assayed, the specialists *B. cacuminata*, *B. cucumis*, and *B. jarvisi* retained strong preferences for their particular hosts. When *B. cacuminata* females were deprived of their preferred host, they did not accept any fruit outside their normal host range. Although *B. jarvisi* females readily accepted non-natural hosts when deprived of their preferred host (*Planchonia careya*), they did not increase the number of eggs laid with increasing periods of deprivation, nor did they accumulate oocytes (as was the case with *B. cacuminata* and *B. cucumis*) (59). In sharp contrast, females of the highly polyphagous species *B. tryoni* did not resorb oocytes and oviposited readily into a previously unacceptable fruit after only 4 days of host deprivation. This author concluded that specialists differed markedly from generalists in the physiological control of oocyte maturation. Whereas in *B. tryoni* there was “no inhibition of oocyte development once the primary follicle had matured,” in *B. cacuminata*, *B. cucumis*, and *B. jarvisi* each ovariole contained only one mature egg. According to Fitt (59), the high egg load and concomitant change in behavior associated with the increase in egg load observed in *B. tryoni* is likely one of the factors that explain why this species quickly accepts non-natural or exotic hosts when deprived of oviposition opportunities.

On occasion, environmental conditions such as severe drought cause fruit trees to

abort fruit early, hinder fruit development to the point where they never ripen, or cause malformed fruit. If, under these circumstances, gravid females with a high egg load exhibiting preadaptations and genetic variance for hosts not used in nature (72) oviposit into malformed (weakened) fruit or fruit from species not previously considered natural hosts, eggs may eclose and larvae may develop into viable adults. Good examples of the latter are the cases of *Bactrocera xanthobodes* infesting malformed ‘Candy Red’ watermelons (*Citrullus lanatus*) in Tonga (159) and *A. ludens* infesting ‘Rocoto/Manzano’ hot peppers (*Capsicum pubescens*) in Veracruz, Mexico (163). Field infestations by *A. ludens* in hot peppers had never been formally reported and were most likely the result of a severe drought causing natural hosts (*Citrus* spp., *Casimiroa edulis*, and *Prunus persica*) to be in short supply or absent. Similarly, Aluja & Birke (2) showed that when one of the preferred native hosts of *A. obliqua* (*Spondias purpurea*) are grown adjacent to an introduced host (*Mangifera indica* ‘Manila’), the latter are not infested. Only when the fruiting season of *S. purpurea* was over did females of *A. obliqua* start infesting mangos. Fitt (58) concluded that a large number of the host records for *B. tryoni* are based on a small number of flies reared from particular fruits on only one occasion, which despite being “suitable for larval development, are nevertheless not normally preferred by females except when alternatives are unavailable.” In sharp contrast, the strong discrimination of *B. cacuminata* against fruit other than *Solanum* (57) represents a good example of absolute discrimination (“when the probability of acceptance of a particular plant species is effectively zero and is not a function of an insect’s motivational state,” 135).

An alternative explanation to unexpected patterns of host use in the field involves the possibility of associative learning. Papaj & Prokopy (123) and others (139) have demonstrated that individuals of many pestiferous polyphagous species can learn and forget (i.e.,

can be reconditioned to accept fruit they had previously rejected). As documented by Prokopy et al. (130), in the apple maggot the propensity to accept a particular fruit and reject others is significantly influenced by recent experience with that fruit.

In sum, host plant choice is not the result of a simple behavior but rather it is the result of a “dynamic hierarchy of several components” (172). Host utilization can vary within an insect species or within an individual in response to changes in the internal physiological state caused by a shortage of preferred hosts (151, 176) or by previous experience with hosts (124). Changes in the internal physiological state may modify preferences by altering the perception of external cues (38) or the sensitivity of peripheral receptors to a particular stimulus coincident with the increase in egg load or by reducing thresholds for particular responses (151). Consequently, the local host utilization patterns of polyphagous insects, which exhibit preference hierarchies, may vary according to the relative abundance of potential alternative hosts when the preferred host is absent (67). This finding led Fitt (57) to conclude that “the resistance of varieties which rely solely on behavioral non-preference may quickly be overcome when cultivated extensively, if the pests become less discriminating in the absence of more acceptable cultivars.”

What happens when females of highly polyphagous fruit fly species are kept in small, crowded cages in the laboratory? If sexually mature females (particularly laboratory-reared ones) are forcibly kept in cages without an egg-laying substrate, their oviposition drive gradually increases and, as time progresses, egg dumping commonly occurs. Females repeatedly attempt to bore into cage walls (138) or stick their aculeus through the screen covering the cage and eventually start dumping eggs. The latter scenario occurs if females are not able to resorb oocytes, as is the case for *B. oleae* (61). Furthermore, flies continuously kept at high densities in small cages

in the laboratory usually lose the ability to recognize fruit surface chemicals because their tarsal receptors break or get blocked by feces and other substances such as food (usually protein/sugar), or they lose sensitivity because of low relative air humidity (157). If a fruit, vegetable, or other object that is not a natural host is introduced into such a cage (e.g., radishes, string beans, strawberries, corn cob, potatoes, or even a pin-pong ball), females will readily accept it as an oviposition substrate (**Supplemental Figure 1** and **Supplemental Video 2**, follow the Supplemental Material link from the Annual Reviews home page at <http://www.annualreviews.org>), which under certain conditions may be an adequate rearing medium from which a viable adult can be produced (16).

HOST SUITABILITY AND THE MECHANISMS USED BY FLIES TO OVERCOME PLANT DEFENSES

Host Plant Resistance to Fruit Fly Attack

Despite the fact that commercially grown fruit tends to be less toxic to mammals than many wild species as a result of artificial selection (80), some fruit into which female fruit flies deposit single eggs or egg masses exhibit antibiosis, which according to Schoonhoven et al. (144) “denotes reduced fecundity, size, or longevity, and increased mortality of the attacking insect.” Earlier, Torre-Bueno (166) had defined “resistance factor” as “any condition in plants that protects them from insect infestation, including structures, chemical substances in the plant, or physiological conditions.” For example, citrus resistance to fruit fly attack has been attributed in part to chemical characteristics of the peel (e.g., essential oils, 5,7-dimethoxy-coumarin, linalool in the flavedo; 78, 141) and to physical properties such as peel thickness (principally flavedo thickness) and peel resistance to puncture

Physiological state: the internal condition of an organism that influences its behavior

Antibiosis: characteristics in plants that inhibit egg eclosion, larval development, or adult fitness

Risk analysis: a process driven by epidemiological principles through which the risk of a pest introduction into any given area, via a commercially traded commodity, is determined

(23, 76, 100). Linalool together with benzyl isothiocyanate (BITC) in papayas helps repel damage by *B. dorsalis* (148), and resins flowing from ducts in the peel of mangos render them partially resistant to the attack of various fruit fly species (29, 90). Finally, in the case of apples, total phenolic content apparently influences degree of resistance (126).

Importantly, there is a correlation between resistance and degree of fruit ripeness (41, 76, 77). One of the best examples of changes in host resistance during maturation was described by Seo et al. (146, 148). In ‘Solo’ papaya orchards, these authors associated maturation with infestation rates and determined that BITC was correlated with papaya ripeness and had inhibitory effects on oviposition by *C. capitata*, *B. dorsalis*, and *B. cucurbitae*.

An effective response to fruit fly eggs by fruit is the formation of hardened calluses around these eggs. These hardenings around oviposition wounds have been reported for *Persea americana* cv. ‘Sharwil’ and ‘Hass’ avocados (3, 102), as well as for lemons (*Citrus limon*) (156). Calluses in conjunction with regenerating tissue (94) may cause egg mortality by asphyxiation, since the eggs of many fruit fly species require about five days to hatch (1). In addition to callus formation, in the case of certain cultivars of avocados and other fruit species [e.g., lychees and longans (75)], there is mechanical resistance (i.e., physical barrier) exerted by the epicarp such that females of some species with short aculei (e.g., *A. fraterculus*, *A. suspensa*, *C. capitata*, and *B. dorsalis*) cannot penetrate and as a result eggs are left on the surface and quickly desiccate (8, 119). According to Jones (91) epicarp thickness appears to be the primary host variable responsible for influencing aculeus morphology, rendering this character of interest for risk analysis considerations.

Fruit (trees) weakened by disease or drought partially lose their ability to quickly form calluses or to regenerate damaged tissue, and this could explain why a few larvae are able to develop (3). Liquido et al. (102),

who worked with ‘Sharwil’ avocados, reported increased susceptibility to fruit fly attack during the dry season. Such findings support the plant stress hypothesis, which postulates that when plants are physiologically stressed, they become more susceptible to attack by herbivorous insects (details in Reference 127).

Mechanisms Used by Flies to Overcome Fruit Resistance to Attack

One means to overcome resistance is to simply lay the eggs away from the toxic barrier, as is the case with the Mexican fruit fly, a notorious pest of commercially grown citrus. A comparison of the ovipositor sheath length (an indirect measure of aculeus size) of other citrus-infesting fruit fly species (e.g., *C. capitata* and some members of the *A. fraterculus* sibling species group) indicated that *A. ludens* had the longest aculeus, allowing females to lay eggs in the nontoxic albedo region and thus avoid the toxic flavedo (23). The long aculeus of *A. ludens* probably represents an adaptation to ovipositing into the seeds of its ancestral host plant *Casimiroa greggii*, also a Rutaceae (9). In contrast, *A. suspensa*, *C. capitata*, and South American populations of *A. fraterculus* lay their eggs only in the flavedo region of the grapefruit peel and cannot avoid toxic essential oils, which cause high egg and larval mortality (23, 76).

Females of *A. ludens* also facultatively adjust clutch size when encountering an inhospitable medium for larval development. This phenomenon was experimentally confirmed by Díaz-Fleischer & Aluja (41), who found that females of this species oviposited larger clutches in firm (unripe) versus soft (ripe) hosts (‘Ataulfo’ mangos were used), a mechanism that significantly increases larval survival in unripe fruit. Alternatively, when a female encounters a poor-quality host, it may reduce the number of eggs laid in a particular plant or can adjust the size or nutritional content of the eggs (14). Yet another mechanism is exhibited by females of primitive species such as *A. cordata*, which apparently probe the fruit until

finding a route that circumvents the toxic laticiferous canals of their latex-producing host (9).

Effect of Cultivar and Time Elapsed Since Harvest on the Degree of Susceptibility to Fruit Fly Infestation

Ample evidence indicates that not all cultivars within a particular species of fruit are equally susceptible to infestation (26), and such information needs to be considered when determining host plant status and calculating risk of fly introductions via infested fruit. Pree (126) and Reissig et al. (136) (and references therein) provided evidence that crab apples and clones thereof (e.g., ‘Fuji’) were completely resistant to the development of *R. pomonella* larvae. Working with a species within the *A. fraterculus* species complex that has recently started to infest apples in Southern Brazil, Sugayama et al. (161) also found that the ‘Fuji’ cultivar was the least infested (11% versus 55% survival when compared with the native hosts within the Myrtaceae). Differential cultivar susceptibility to fruit fly attack has also been shown in citrus (8 and references therein, 100), guavas (133), and mangoes (29, 83).

Egg eclosion and larval development/survival differ, many times radically, in unpicked versus picked fruit (3, 26). Larval mortality is significantly greater in fruit naturally attached to the tree compared with picked fruit (3, 26), a phenomenon most likely related to the fact that fruit condition changes substantially as soon as it is removed from the tree (113, 167). The ability of flies to artificially infest picked fruit under laboratory conditions is intimately related to the time since harvest of the fruit used in bioassays, indicating that perhaps resistance mechanisms become less efficient or cease to function. For example, Oi & Mau (119) found that infestations ranging between 0% and 30% were obtained from ‘Sharwil’ avocados exposed at 0–2 h postharvest but that the numbers increased to 66.7%–100% at 3–7 h postharvest. Simi-

larly, Aluja et al. (3), found that ‘Hass’ avocados exposed under highly artificial conditions to gravid *A. ludens* females 3 and 72 h postharvest did not yield any larvae. In contrast, larval development was observed (albeit in small numbers) in fruit exposed 24 and 48 h postharvest. Notably, none of the fruit exposed to three other *Anastrepha* species (*A. obliqua*, *A. serpentina*, and *A. striata*) yielded any larvae (3). This shows that resistance mechanisms are dynamic and can be species specific.

A STEPWISE APPROACH TO EXPERIMENTALLY DETERMINE FRUIT FLY HOST STATUS INCORPORATING RISK ANALYSIS CONSIDERATIONS TO PROBLEM SOLVING

In this section we provide details on our proposed flow diagram (Figure 1) and a series of definitions that will hopefully aid scientists and regulatory officials in following science-based decision-making processes with respect to fruit fly/host plant designations. Our scheme recognizes the pioneering work by Willard et al. (177), who 78 years ago proposed a stepwise approach similar to that of Cowley et al. (36). Importantly, we propose reversing the order of events by starting where Willard et al. (177) and Cowley et al. (36) left off, that is, in the field.

Our flow diagram (Figure 1) is based on the fundamental aspects that drive host use by insects and key life-history parameters discussed above (e.g., host breadth, number of generations). We identify risk analysis and systems approach procedures to help strengthen the host-designation protocol to minimize errors in interpreting results (i.e., minimize the possibility of false positive and false negative results) and to minimize the risk of accidental introductions into importing countries. Highly polyphagous, pestiferous fruit fly species such as *C. capitata*, *B. carambolae*, *B. dorsalis*, *B. papayae*, *B. tryoni*, *A. ludens*, and *A. suspensa* should be considered prime

candidates to be submitted to our experimental protocol.

Operational Definitions

We define a natural host as a fruit taxon that has been unequivocally reported to be infested under totally natural field conditions (i.e., nothing is manipulated; details under “Guidelines for Reporting Fruit/Vegetable Infestations by Fruit Flies in Nature,” follow the Supplemental Material link from the Annual Reviews home page at <http://www.annualreviews.org>). A non-natural host is a fruit taxon that has never been unequivocally reported to be infested under unmanipulated field conditions but has reliable experimental evidence that it could provide adequate properties to be infested and produce reproductive adults under manipulated (artificial) laboratory conditions.

These two host classes have been discussed under a variety of terminologies (58, 114, 171) that incorporate the fundamental evolutionary, behavioral, physiological, genetic, and ecological underpinnings of host use discussed above: fundamental (conditional, potential, artificial) = non-natural and realized = natural host range. These concepts are derived from the pioneering conceptualization by Hutchinson (85) on the nature of the niche: fundamental and realized niche, with Fitt (58) being the first to introduce them into the fruit fly literature. Fitt (58) defined fundamental (he used the word “potential”) host range as “the entire range of plants on which development is possible and that will be influenced largely by the physiological and morphological adaptations of larvae which allow them to utilize the available nutrients and detoxify or avoid toxic secondary compounds.” More recently, van Klinken (171) defined the fundamental host range as follows: “the absolute limits to an insect’s host range, which circumscribe fundamental host range, are constrained by such factors as its metabolic and sensory capabilities, physical limitations and behavioral programming.” Fitt (58) defined

realized (i.e., natural) host range as “the actual range of plants on which larval feeding occurs which is influenced much more by the behaviors which allow adult females to locate, recognize and accept different plants for oviposition,” while van Klinken (171) defined the term (under “field host range”) as “what actually happens in the field” or, in the words of Hanson (79), “host plants are those on which the animal completes normal development in nature.” Van Klinken (171) goes on to state that “under field conditions, the realized host-range is frequently a subset of the fundamental host-range. That is, insects often accept or use only a proportion of those that they are capable of.”

Nichols et al. (114) indicated that the “fundamental host range” is genetically delimited and that the realized host range “is constrained by environmental influences, which include physical (e.g., geographic barriers, climatic tolerance) and biological (e.g., competition, predation) factors.” For example, in the case of a univoltine fruit fly, lack of coincidence between adult emergence patterns (in temperate regions driven mainly by diapause schedules) and fruiting phenology will limit the use of a host that is perfectly suitable for larval development. An example of this situation providing quarantine security in certain regions has been described by Yokoyama & Miller (180, 181) for the walnut husk fly (*Rhagoletis completa*), which infests the walnut *Juglans regia*. This species can infest certain stone fruits grown in the San Joaquin Valley counties of California. However, these stone fruits mature and are harvested before the husk fly populations emerge, so the stone fruits are effectively phenologically isolated from the walnut husk fly pests (180).

As noted by van Klinken (171), the fundamental host range can be determined for each developmental stage of the insect [e.g., egg, larva (each instar if necessary), pupae, and adult] or for the life cycle of the insect. In the case of adults, van Klinken notes that the concept can be refined to the level of determining the limitations (constraints) faced at each step

in the catenary process of host finding and acceptance. The fundamental host concept can be useful when comparing the ability to develop in a potential or conditional host by several species in one genus, as was the case with the ‘Hass’ avocado and forced oviposition by *A. ludens*, *A. obliqua*, *A. serpentina*, and *A. striata* females. Of these, only *A. ludens* larvae reached the pupal stage, with no adult emerging from the undersized pupae (6). That is, such a fruit would fall into the category of fundamental or conditional/artificial host for the egg and larval stages, but it would not be part of the natural (realized) host range. We believe that adopting the fundamental versus realized host range concepts will allow regulatory and quarantine officials to decide, case by case, the level of resolution required for calculating sample sizes and risk (63).

For the sake of completeness in this review, we note that Hennessey (82) proposed host definitions for regulatory applications that fully coincide with our proposed scheme: fruit fly nonhost, fruit fly host, conditional fruit fly host, and conditional fruit fly nonhost.

Field Surveys and the Need for Accuracy When Reporting Fruit/Vegetable Infestations by Fruit Flies in Nature

Once we have given consideration to the fundamentals of host use by fruit flies and have access to clearly stated operational definitions, the next critical step is to gather credible information in the field based on past, current, and future surveys. Such surveys allow researchers to determine whether any given fruit (or vegetable) is a natural host or not. This dichotomy represents the crux of our proposal.

Given that phytosanitary considerations are frequently the major barriers to trade in fresh fruit/vegetables, the need for accuracy in reporting fruit fly/host associations (and expunging dubious records) has taken a much more significant role in market access

than during periods prior to free-trade agreements. It is thus critical that generic names such as avocados, mangos, oranges, or apples be avoided in this context. For example, many times authors do not refer to *P. americana* but simply to “avocados” and almost never specify the expert plant taxonomist who identified the *Persea* species and cultivar they worked with or the expert fruit fly taxonomist who identified the fruit fly species involved and kept voucher specimens. Considering that the genus *Persea* is divided into the subgenera *Persea* and *Eryodaphne*, that worldwide there are more than 85 species of *Persea* (145), and that within *P. americana* there are more than 500 cultivars (96) of West Indian, Guatemalan, or Mexican origin (145), the word “avocado” becomes meaningless, at least in the context of fruit fly/host plant determination/designation. The same applies to mangos, oranges, apples, and many other fruit species in which case there are also hundreds of cultivars (142).

Furthermore, many times there is a problem with respect to the quantities of fruit that were sampled and the degree of infestation reported because there is a significant difference between one larvae/fly per fruit and one larvae/fly per 10, 100, or 1000 pieces of fruit (31, 63). For example, Uchôa & Zucchi (169) collected 50 pieces of fruit of *P. americana* in Mato Grosso, Brazil, weighing on average 17 g. From this sample, they recovered 120 adult flies (Tephritoidea), of which 82.5%, 16.7%, and 0.8% (one specimen) were uliids (formerly otitids), flies in the genus *Neosilba* (Lonchaeidae), and *A. striata* (Tephritidae), respectively.

Finally, we need to eradicate the problem caused by chains of citations referring to an originally flawed report. For example, Rust (140), who had no hard evidence whatsoever [i.e., information provided was obtained from a government list or based on supposed personal knowledge: “. . . following fruits which the author *knows to be infested*. . .” (italics added)], has ever since been cited and recited without any scrutiny [e.g., Costa-Lima (32)

citing Rust (140) and then Blanchard (24) citing Costa-Lima (32), who in turn was cited by Turica et al. (168) and so on]. Even more damaging is that this misinformation ended up in annotated host catalogs (174) and electronic host plant databases (115, 116) that are used worldwide. Instead of legitimizing such flawed records by incorporating them into respectable databases, they should, in our opinion, be expunged from the record or not cited. We propose a series of strict guidelines for future natural host plant reports that should help solve these types of problems (follow the Supplemental Material link from the Annual Reviews home page at <http://www.annualreviews.org>).

Quarantine Treatments, Risk Analysis, and Systems Approach to Address Issues Related to Natural Hosts

Once we have rigorously determined that a particular fruit or vegetable is naturally infested under unmanipulated field conditions, and have provided adequate information on the ecological context in which such infestations occurred, a series of steps follow. In the flow diagram (**Figure 1**) both the natural host and the non-natural host dichotomies ultimately end with options that include additional research, quarantine treatment, and risk analysis and systems approach. For example, continuing research is advisable to answer questions such as the similarity of volatile profiles or contents of secondary metabolites in different cultivars of a particular fruit species infested in the field. Additionally, we need to apply risk analysis, systems approach, and quarantine treatment procedures should any given country be interested in importing a certified natural fruit fly host. For example, a series of postharvest treatments have been approved by the USDA APHIS as quarantine treatments (170), but these can vary depending on the particular circumstances or commercial partners involved. Relevant reviews of methods for achieving and evaluating quar-

antine security by quarantine treatments and combinations of treatments and population control, in connection with host status, can be found in References 63, 64, 104, 106, 107, 125, and 150.

The purpose of regulatory quarantine actions is to prevent transport of infested commodities and to reduce the risk of introducing pests into pest-free areas. The term risk is defined as the “probability that an outcome will occur times the consequence or ‘level of impact’ should that outcome occur” (93). Regulatory agencies and international working groups have addressed the issue of risks associated with international trade of fresh commodities. A summary of agreements under the International Plant Protection Convention (IPPC) has been reviewed by Devorshak & Griffin (39). Documents published by the IPPC that describe the standards for controlling spread and introduction of plant pests are found in a series of International Standards for Phytosanitary Measures (86).

The process of pest risk analysis for exotic pests as trade issues is described in References 49–51. Devorshak & Griffin (39) summarize this process as three stages: (*a*) initialization, in which pests are identified as potential hazards; (*b*) risk assessment stage, in which the probability and consequences of establishment are evaluated; and (*c*) risk management stage, in which options for eliminating or reducing pest risk are evaluated and recommendations are developed. Problems with the initialization stage can be due to unreliable literature and taxonomic questions concerning whether evaluations should be limited to a specific cultivar or the plant species in general. For the risk assessment stage, the issue of host status for quarantine consideration has occasionally been highly contentious. The type of research proposed here will hopefully clarify matters. However, this stage extends past the issues of pest biology and many times involves economic, social, and political considerations that inevitably creep into the process of host plant status designation. Dealing proactively with these types of pressures is as important as

determining the host status of a given commodity following scientifically acceptable methods because science-based decision-making processes represent the only acceptable mechanism to resolve any dispute on this matter. Risk management returns options including those covered by components of the systems approach.

Oviposition Bioassays Under Forced, Artificial Conditions

If there is credible information indicating that a particular fruit or vegetable species or cultivar is not naturally infested in the field (**Figure 1**, non-natural host box), then a series of tests are needed to determine whether sexually mature, gravid females will respond to the volatiles and lay eggs into a particular fruit (considering such factors as fly condition, degree of ripeness, cultivar, and ecological context in which fruit is grown). We also need to experimentally determine if the commodity allows eggs to hatch and sustains larval development, if such larvae are able to pupate, and most importantly, if emerging adults are able to reach sexual maturity and produce viable progeny. In other words, to determine the fundamental host range (171). Note that any test under highly artificial conditions inevitably entails the danger of eliciting aberrant behaviors in flies (**Supplemental Figure 1** and **Supplemental Video 2**), potentially leading investigators to reach flawed conclusions at least from a biological and regulatory perspective (i.e., false-positive and false-negative results). The non-natural conditions in these tests can lead to manipulations such as early removal of larvae from a poor host (requiring greater development time than a preferred host), resulting in low or failed pupation and lack of adult emergence (false-negative results).

There are three approaches to forced, artificial oviposition bioassays: (a) field cages or greenhouses covering entire fruit-bearing trees (**Figure 2a**), (b) enclosed fruit-bearing branches (3, 177) (**Figure 2b**), and/or (c)

wooden or Plexiglas cages containing harvested fruit (3, 177) (**Figure 2c**). We strongly recommend avoiding tests with picked fruit because, as noted above, fruit condition and the volatile profile emitted by the fruit change



Figure 2

(a) Field cage enclosing entire fruit-bearing guava tree, which is an ideal experimental arena to conduct foraging behavior tests. (b) Fruit-bearing branch (*Mangifera indica* 'Manila') enclosed by tulle screen into which fruit fly females can be artificially released. (c) Plexiglas cage into which harvested fruit have been introduced to conduct oviposition tests under highly artificial laboratory conditions. Ideally, fruit should be naturally attached to peduncle and branches should have foliage naturally attached to them.

radically as soon as it is removed from the tree (113, 167). As a result, infestation records under such highly artificial conditions contribute only marginally to our understanding of the biology of fruit fly host use and, most importantly, contribute little to the process of host plant status determination/designation because such records only confirm that we are dealing with an artificial host. Tests under the first two approaches are run in the field, and the last approach is usually run in the laboratory, although cages can be placed in the field under the shade of a tree. All tests can be run under choice (i.e., known natural host plus test commodity offered to females simultaneously) or no-choice conditions. Preferably wild flies should be used, although on occasion one needs to resort to laboratory-reared insects as wild flies are not always available in large enough numbers or when comparing the performance of wild versus laboratory-reared flies is of interest (see Reference 6 for further details). For a review of potential problems with choice or no-choice tests, we refer the reader to References 108 and 165. Only the first approach allows for detailed observations on foraging behavior.

When females of polyphagous species, especially those reared in the laboratory, are exposed to fruit under no-choice conditions or even under choice conditions, particularly in field enclosed, fruit-bearing branches or in laboratory cages, they will lay eggs into the test fruit (or vegetable), which allows for the determination of larval development, pupation, and adult emergence (and performance). Oviposition activity under highly artificial conditions can be observed because we have artificially bypassed critical distance cues that a foraging female would use to detect its host under field conditions. Many times the eggs are laid into fruits that are never infested in nature. In addition, females will sometimes attempt to bore and lay eggs, but are unable to do so because they cannot puncture the epicarp of the fruit. As a result, eggs are only partially inserted or left on the fruit surface, causing rapid desicca-

tion (a real limit of the fundamental host range).

While running the battery of tests described above, there are a series of critical methodological considerations that need to be taken into account (details in References 3, 8, 82, 108, 153, and 171). Owing to space restrictions we cannot address them here in detail. Nevertheless, given their potential influence on the outcome of bioassays, the following factors should be adequately controlled: (a) physiological and physical state of the insect, in particular egg load and concomitant level of motivation, degree of hunger (only well-fed insects should be used), age (spread in age classes of insects used in bioassays should be reduced to a maximum of 5 days), low level of wear or damage to critical structures (tip of aculeus, antennal and/or tarsal receptors, wings); (b) use of wild, wildish (i.e., wild genotype reared for a defined number of generations in natural hosts under laboratory conditions), or laboratory-reared flies stemming from a defined geographical region to sidestep local adaptation issues; (c) degree of ripeness of fruit, measured by dry matter or sugar content; (d) health (vigor) of fruit-bearing tree; (e) consideration of potential variability in both the commodity and experimental fruit fly populations over entire growing areas and periods (details in Reference 82); (f) consideration of differences among cultivars; (g) repetition of experiments during wet/dry or warm/cold seasons that should be adequately replicated. Furthermore, we suggest adopting the definitions by Singer (153 and references therein) for terms widely used in tests aimed at determining host range or host status.

A number of experimental factors can affect the outcome of artificial, laboratory or caged tests in the field. For example, Oi & Mau (119) demonstrated that tree-attached 'Sharwil' avocados could be infested by *B. dorsalis* and *C. capitata*, whereas Armstrong et al. (13) reported that no infestation by these species occurred. Discussion by Oi & Mau (119) identified differences in the number of females released [35 (119) versus 5

females per cage (13)] and exposure time to the fruit [3 days (119) versus 24 h (13)]. Methods of holding and evaluating fruit can also vary among tests. For example, Aluja et al. (3) removed larvae from both 'Hass' avocados and the preferred hosts (mango, sapodilla, guava, and grapefruit) in their caged tree tests after 22 days (when fruit were totally rotten and all living larvae had exited). In contrast, Ohto et al. (118) showed that development time (egg to adult) for cage-infested mangos and grapefruit was 36 days and for their "avocado" samples the development time was 52 days. Bush (27) and Willard et al. (177) avoided these problems by holding fruit until larvae exited (as described in Reference 3) and then holding pupae for adult emergence.

QUARANTINE TREATMENTS, RISK ANALYSIS, AND SYSTEMS APPROACH TO ADDRESS ISSUES RELATED TO BOTH CONDITIONAL AND NATURAL HOSTS

Once oviposition bioassays are completed, the following dichotomy will emerge: The insect is or is not able to complete its life cycle. If the insect cannot, then the commodity is labeled a nonhost and what follows would be screening every 15–20 years to detect mutations in fly populations that would allow certain individuals to develop in a fruit or vegetable (and cultivars thereof) previously unfit for it. If the insect can complete its life cycle, then the commodity is labeled a potential, conditional, or artificial host. If such is the case, four possible routes emerge: (a) additional research to determine degree of suitability, particularly in the case of cultivars; (b) risk analysis including pathway analysis; (c) development of quarantine treatments, and (d) consideration of the economic and political impact of the possible importation of the commodity if there is opposition by stakeholders but guaranteeing that final rulings are science based and not politically motivated. The issue of differential cultivar susceptibility is a contentious one

already prompting a ruling by a World Trade Organization Dispute Panel in 1998. The ruling came upon the request of U.S. plant protection authorities fending off requests by the government of Japan that every single cultivar of peach and tangerine be tested for effectiveness of fumigation procedures as treatments against the codling moth (178).

Baker et al. (17) developed a maximum pest limit concept for determining the level of fruit infestation that would be permitted for various commodities combined with a postharvest treatment. They based their detections of infested fruit on the numbers of fruit cut for inspections, but they did not consider the accuracy of the inspection process (false negatives with infested fruit not detected when cut), which, as shown by Gould (74), can be low (i.e., most fruit below 50% efficacy).

A series of other publications have approached methods of calculating inspection requirements, distributions of pests among fruit, and estimates of numbers of pests passing through ports of entry. Mangan et al. (103), who used *A. ludens* infestation rates in various citrus and mango cultivars from field collections, showed that for unmanaged populations, the infestation rate allowed infestation levels high enough so that in commercial shipments even with the Probit 9 treatment, more than two survivors would be introduced and, using the equations of Couey & Chew (34) to evaluate levels of inspection, large samples of fruit would have to be cut at ports of entry to detect these survivors.

Yamamura (179) used infestation data from Mangan et al. (103) to examine and predict fruit inspection requirements for shipments entering Japan. He assumed a beta distribution of pests among fruit rather than a Poisson distribution, and although it was not shown that this distribution is the best for this type of calculation, it does address the non-Poisson problem. Precision of the inspection system (74) is also a crucial factor in Yamamura's system.

Follett & Hennessey (63) have recently attempted to develop calculation criteria for

determining confidence limits and sample sizes for evaluating nonhost status. The calculation methods used by Couey & Chew (34), Landolt et al. (97), and Baker et al. (17) applied in this paper are all based on pests distributed among fruit or commodity units as a Poisson function. However, nearly all data for infestation distributions among fruit show a negative binomial distribution, with the variance in pests among fruit far exceeding the mean (103).

Mangan & Sharp (106) corrected the equations of Seo et al. (147) to propose methods of evaluating multiple quarantine treatments or combinations of treatments, as not all commodities respond equally well. For example, when Mangan et al. (107) tested quarantine treatments for three citrus commodities (grapefruit, 'Valencia' oranges, and 'Dancy' tangerines) using the identical *A. ludens* strain, equipment, rearing facilities, and technicians, they discovered that mortality models (Probit, Logit, Cloglog) indicated that mortality distributions in the dose response tests differed among the fruit types.

In many of the cases discussed above, especially Jang & Moffitt's (89) proposal of the systems approach, the host status differences are included as important components of infestation probability that can determine the numbers of pests entering a quarantined habitat. Sequeira (149) has illustrated the pathway for introduction of pests by movement of commodities and establishment of pests as a series of components. In his expression, probability of pest entry establishment includes the size of the shipment, probabilities of fruit infestation and pest surviving to the fruit harvest, possible selection of fruit at harvest, postharvest treatment, mortality during packing (culling of fruit) and shipment, and arrival in a suitable habitat and locating a suitable host under suitable conditions in that habitat. Sequeira (149) further notes that the pathway components and their probability distributions must have common units and be independent. He therefore cautions against methods

that "simplistically combine" them into a model.

Superficially, the use of a systems approach to quarantine security would appear to have lower security than a system of certifying producers and having postharvest treatments. Reviews of the literature, however, show that importation systems having Probit 9 levels have failed to prevent pest entry. Mangan & Hallman (104) give several examples of heat treatments that have failed to provide quarantine security. Failures of fumigation systems, though reported less frequently, also allow fruit flies to survive. Perhaps the most contentious recent failure was the finding of surviving Mediterranean fruit flies in 'Clementine' oranges exported from Spain to the United States (55). This failure occurred three years after the massive, repeated outbreaks of Mediterranean fruit flies in Florida, so public attention was significant. The imposition of a more rigorous systems approach, in addition to better regulation of the detection programs and more efficient population control (105) and less costly eradication options such as preventive sterile fly release programs (48) operating in high-risk areas in Florida and California and the area-wide fly-free area for Mexican fruit fly in south Texas, suggests that the newer multi-approach systems to stop entry of exotic fruit flies are indeed preventing outbreaks that typified the 1990s. However, if the regulatory combination of detection and action are not vigilant against a pest species, even monophagous fruit fly pests such as the olive fruit fly (*B. oleae*) may enter and establish populations.

CONCLUSIONS AND FUTURE DIRECTIONS

This review was designed to provide a conceptual basis and to link the biology of host selection and utilization by fruit flies to actual protocols and interpretations for regulatory management. Fruit fly/host plant relationships (designations) represent a highly

complex phenomenon. Any effort to determine the host status of a particular commodity and the potential risks to agriculture will be strengthened if the problem is viewed from as wide a perspective as possible and is solved by rooting all experimental and technical procedures in the biology and behavior of the fruit fly species involved and by developing flexible systems approaches applied on a case-by-case basis. **Figure 1** depicts recommended survey and testing procedures along with feedback loops for continuous monitoring that should aid science-based decision-making processes in the future.

How could the study of fruit fly/host plant relationships be advanced in the future? In our opinion, the only sensible route is to sponsor an uninterrupted effort aimed at gaining deeper insight into the fundamentals that drive host use by fruit flies because, as aptly noted by Cowley et al. (36), “host status should be regarded as a continuously evolving phenomenon.” In this respect, we need to study the genetic mechanisms underlying host use by fruit flies along the lines outlined by Via

(172) for other herbivorous insects. For example, we need to measure the overall genetic variability in characters associated with host use, identify loci associated with host preference, study the possible inheritance of preference, and analyze the interaction between genetic and environmental components possibly constraining host expansion. A strong genetic basis would in turn allow researchers to study the physiological processes underlying metabolic and behavioral responses to plant-derived cues (adult flies) and allelochemicals (larvae). In this respect, molecular approaches to resistance (56) and the emerging field of nutrigenomics (33), coupled with recent discoveries on plant defense mechanisms (25), will undoubtedly help us better understand and, as a result, engineer host resistance to fruit fly attack. For example, by investigating how nutrition alters global gene expression patterns, one could rapidly evaluate how insects respond to various fruit, or discover the resistance mechanisms exhibited by, for example, *P. americana* ‘Hass’ when artificially infested by *A. ludens* (3).

SUMMARY POINTS

1. Given the economic and political impact of the issue of host plant status determination at the international level, here we present a conceptual and methodological framework that can aid scientists, regulatory officials, policy makers, politicians, and stakeholders in solving current and future disputes over the status of particular plants as fruit fly hosts.
2. Fruit fly/host plant relationships represent a highly complex phenomenon. Any effort to determine the host status of a particular commodity will be strengthened if the problem is solved by rooting all experimental and technical procedures in the biology of the fruit fly species involved and by developing flexible systems approaches applied on a case-by-case basis.
3. Host plant choice is not the result of a simple behavior but rather represents a dynamic hierarchy of several components. Host utilization can vary within an individual in response to changes in internal physiological state (e.g., egg load) caused by a shortage of preferred hosts or previous experience with hosts. Consequently, in the absence of their preferred host, in polyphagous insects which exhibit preference hierarchies, local host utilization patterns may vary according to the relative abundance of potential alternative hosts.

4. Some of the most important factors that influence fruit fly oviposition behavior and that can potentially lead a female to lay eggs into a fruit outside of its natural host range in nature or in experiments under artificial, laboratory conditions are: host quality, genetics, learning, potential fecundity, ovarian dynamics (e.g., egg load and concomitant oviposition drive, ability to resorb oocytes), aculeus wear, female age, social context (e.g., presence of conspecifics), chemical context (i.e., presence of fruit volatiles and sexual pheromones), and individual variation in oviposition decisions.
5. When analyzing fruit fly diet (host) breadth it is useful to treat it as a continuum and continuously evolving phenomenon as there is great degree of variability among individuals and populations of a particular species and also host ranges shrink and expand over time. On the other hand, absolute limits can be also found with some plants exhibiting complete resistance to infestation.
6. Among natural hosts (those found infested under totally natural field conditions) not all are equally suitable for larval development and even among non-natural hosts, some are acceptable under particular conditions (treated as conditional/potential/artificial host in this review).
7. Host plant designations should be science based and not be tainted by political/economic pressures by stakeholders.
8. Host status evaluations have been recognized as key factors in the systems approach that incorporates “all pre- and postharvest factors affecting infestation and establishment of pests into an integrated system to meet quarantine requirements (and thus satisfy conditions of the pest risk analysis).”

DISCLOSURE STATEMENT

RLM: As a Research Entomologist for USDA-ARS, I make evaluations of host status for fresh commodities subject to fruit fly infestation. I serve on teams writing documents for USDA and FAO (the IPPC pamphlets), describing methods of evaluation and standards for classification of commodities and Tephritidae pests. I advise both USDA and foreign agriculture agencies concerning the research approaches and regulatory decisions for actions concerning host status, needed quarantine actions, and the systems approach for import and export of fresh commodities. In this paper, Martín Aluja and I combined our knowledge and experience to review the biology of host status for both research and regulatory decision making.

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Guidelines for Reporting Fruit/Vegetable Infestations by Fruit Flies in Nature

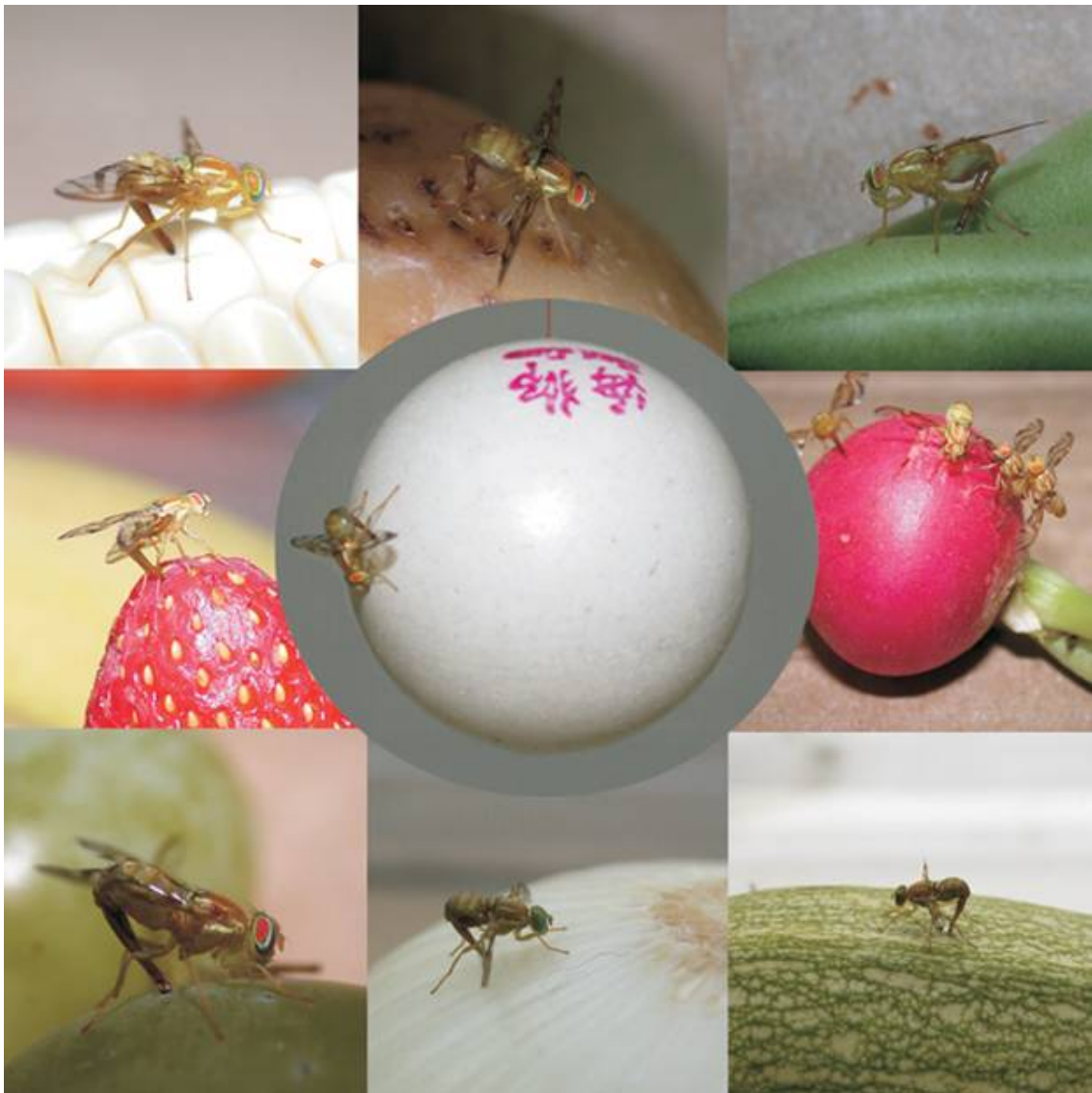
1. Infestation occurring under totally natural field conditions, not artificial, laboratory ones (i.e., nothing is manipulated). Given inherent variability among trees and fruit growing regions and macroclimatic effects on infestation patterns, it is recommended that surveys aimed at determining natural host status and size of adult populations, be carried out a minimum of five consecutive years and run over the entire geographical range of distribution of the particular fly species to account for local population effect (details in main body of review). In addition to fruit sampling efforts, traps should be placed in the vicinity to ascertain presence of adult flies and population size over time, but extreme precautions should be taken to avoid reporting purported fly-host associations based on adults captured in traps that were hung in a fruiting tree, but not reared from fruit (e.g., 5).
2. Quantitative information on levels of infestation (i.e., larvae per kg of fruit, proportion of fruit infested in sample), sample weight, larval and pupal mortality, proportion of adult emergence (i.e., of total number of pupae how many adults emerged), basic demographic parameters such as maximum adult longevity, sex ratio, and if at all possible female fecundity [details on sample processing in (4) and on demographic methods in (3)]. At least 33% of the sampled fruit should be individually weighed and maintained (i.e., one fruit per container) to determine the mean number of larvae per fruit and infestation distributions (a critical consideration when performing risk assessments). It is critical to specify if larvae detected inside fruit ended up pupating, and if such was the case, if adults emerged and if these were able to reach sexual maturity and produce viable progeny. Simply finding larvae inside fruit does not represent a valid host record as many times larvae die before pupating, adults fail to emerge from pupal cases (e.g., 1), die shortly after emergence (e.g., 2) or are not tephritids but rather lonchaeids (decomposers; e.g., 6).
3. Information on plant part used by the fruit fly female to lay its eggs (e.g., flower, seed, stem) and for larvae to complete development (e.g., seed, pulp). It is critical to specify if samples were collected from tree or ground.
4. Information on fruiting phenology of host plant if possible over a five year period to incorporate information on the typical variability in these cases. In addition, all collecting sites should be geo-referenced for verification purposes and authors should provide information on climate (e.g., mean annual temperature and rainfall, seasonality (dry vs wet season of year), soil type, etc).
5. Information related to health status of tree from which fruit were collected. Ideally, if the tree was diseased, the disease agent should be identified. Also, the authors should note conditions such as heat or water stress as they can help to properly contextualize the infestation report.
6. Fly species identified by internationally recognized authority (i.e., expert taxonomist formally acknowledged by citing name and affiliation and by providing the editor of the journal where the finding ends up being reported, a copy of the correspondence with the expert involved). Ideally, high quality digital pictures should be taken and archived and identification should be based on both immatures (e.g., larvae, pupal cases) and adults of both sexes.
7. Plant species *and cultivar* identified by internationally recognized authority (i.e., expert taxonomist formally acknowledged by citing name and affiliation and by providing the editor

of the journal where the finding ends up being reported, a copy of the correspondence with the expert involved).

8. Voucher specimens deposited in the collections of internationally recognized expert insect and plant taxonomists and also in the collection of the host institution if it applies. Voucher specimens should include well preserved third-instar larvae, pupal cases from which the adult fly emerged, adult flies and plant material (leaves, flowers and fruit). In addition to the latter, high quality digital pictures should be taken and archived including evidence of the fruit processing methods and handling of the insects (immature and mature stages). Whenever possible, DNA extractions should be made. If such is not possible, a proportion of the voucher specimens should be equally divided into sub samples kept in a) 99-100% ethyl alcohol and b) minus 80°C (an ultra freezer) for future genetic determinations.
9. Record should be invariably published in a peer refereed, indexed international journal [i.e., appearing in the listings of the Institute for Scientific Information, Inc.® (ISI®)], so that experts be given the opportunity to validate it before it enters the mainstream literature.

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Supplemental Figure 1.

Aberrant ovipositional activity by *Anastrepha ludens* females caused by extreme artificial experimental conditions. Females ovipositing into a strawberry, string bean, onion, corn cob, radish, potato, grape, zucchini, ping-pong ball, events that would almost certainly never occur in nature. Link is provided to a filmed sequence of an *A. ludens* female ovipositing into a ping-pong ball (Supplemental Video 2).