Confidence Limits and Sample Size for Determining Nonhost Status of Fruits and Vegetables to Tephritid Fruit Flies as a Quarantine Measure

PETER A. FOLLETT[1] and MICHAEL K. HENNESSEY[2]

U.S. Pacific Basin Agricultural Research Center, USDA–ARS, P.O. Box 4459, Hilo, HI 96720

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ABSTRACT Quarantine measures including treatments are applied to exported fruit and vegetable commodities to control regulatory fruit fly pests and to reduce the likelihood of their introduction into new areas. Nonhost status can be an effective measure used to achieve quarantine security. As with quarantine treatments, nonhost status can stand alone as a measure if there is high efficacy and statistical confidence. The numbers of insects or fruit tested during investigation of nonhost status will determine the level of statistical confidence. If the level of confidence of nonhost status is not high, then additional measures may be required to achieve quarantine security as part of a systems approach. Certain countries require that either 99.99 or 99.9968% mortality, as a measure of efficacy, at the 95% confidence level, be achieved by a quarantine treatment to meet quarantine security. This article outlines how the level of confidence in nonhost status can be quantified so that its equivalency to traditional quarantine treatments may be demonstrated. Incorporating sample size and confidence levels into host status testing protocols along with efficacy will lead to greater consistency by regulatory decision-makers in interpreting results and, therefore, to more technically sound decisions on host status.

KEY WORDS quarantine statistics, phytosanitary treatments, fruit flies, probit 9

World trade in fruit and vegetable commodities continues to grow (USDA NASS 2006). As agricultural trade between countries increases, the risk of introducing exotic fruit flies (Diptera: Tephritidae) into new areas where they may become plant pests will increase. The introduction of a new fruit fly quarantine pest is extremely costly due to increased crop damage, control programs, and quarantine restrictions on trade (Carey and Dowell 1989, Paull and Armstrong 1994). Quarantine treatments are phytosanitary measures that eliminate, sterilize, or kill fruit flies in exported commodities, thereby reducing the likelihood of their introduction into new areas according to the acceptable level of quarantine security imposed by the importing country. Although a single postharvest quarantine treatment (e.g., fumigation, hot water immersion, and irradiation) applied to a commodity is still the most commonly used approach (Paull and Armstrong 1994), a range of alternative options, including nonhost status, are being applied at an increasing rate (Liquido et al. 1995b, Aluja et al. 2004, Follett and Neven 2006).

For some countries, commodities, and pests, probit 9 treatments have traditionally been required to provide an acceptable level of quarantine security (Liquido et al. 1995b, Follett and Neven 2006). The probit 9 reference originates from the statistical method (probit analysis) used for deriving the dose–response relationship (Baker 1939). A response at the probit 9 level results in 99.9968% efficacy. The required response may be mortality, sterility, or prevention of maturity. The United States Department of Agriculture (USDA) has used 99.9968% efficacy as the basis for approving many treatments as meeting quarantine security requirements, particularly for tephritid fruit flies, but for other high-risk pests as well.

Confidence levels have not been reported consistently along with efficacy in nonhost status testing. As a result, it has been difficult to demonstrate equivalency between a postharvest treatment and nonhost status as alternative measures. In both cases, the goal is the same—to demonstrate that insect survival rates are sufficiently low to ensure quarantine security at a specified level of precision.

This article outlines how to determine confidence levels based on the sample size used during nonhost status testing so that its equivalency to traditional quarantine treatments can be demonstrated. We apply the statistics presented by Couey and Chew (1986) to cases of nonhost testing published in the literature to illustrate how confidence levels associated with the efficacy can be measured and reported. Although fruit

[1] Corresponding author, e-mail: pfollett@pbarc.ars.usda.gov.

[2] USDA–APHIS–PPQ, Center for Plant Health Science & Technology, Plant Epidemiology and Risk Analysis Laboratory, 1730 Varsity Dr., Suite 300, Raleigh, NC 27606.

fly hosts may include fresh fruits and vegetables, for simplicity we use the term fruit hereafter.

Nonhost Status. Armstrong (1986, 1994) defined a fruit fly host as a fruit or vegetable on which a female deposits eggs, the eggs hatch into larvae, and the larvae feed and develop to form viable pupae from which adults emerge. Therefore, if the insect cannot completely develop to form viable adults that are capable of reproduction, then the plant is a nonhost. A nonhost has some inherent characteristic that prevents attack by the pest or prevents the pest from completing its life cycle (Painter 1951). Nonhost status can be used without an additional quarantine measure to achieve quarantine security if it is determined that the regulatory pest will not be introduced with the host into the import area. A commodity may be a nonhost for all or part of its growth cycle, and certain varieties of a commodity may be nonhosts, whereas other varieties are not (Greany 1989, Armstrong 1994). For example, Hawaiian 'Cavendish' bananas are approved for export to the United States mainland from Hawaii as nonhosts for Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and oriental fruit fly, *Bactrocera dorsalis* (Hendel), when harvested in the mature green stage and free of blemishes, although ripe fruit are preferred hosts (Armstrong 2001).

Probit 9 Quarantine Treatments and Alternatives. To achieve 99.9968% response (probit 9) at the 95% confidence level, a minimum of 93,613 insects must be tested with no survivors (Couey and Chew 1986). The large experimental sample size ensures the importing country that acceptable confidence levels accompany a high level of efficacy. The 95% confidence level means that we have a one in 20 chance that the true survival is greater than the observed survival. Confidence levels are typically reported along with efficacy when new postharvest quarantine treatments are developed. Quantitative methods have been developed to calculate the number of test insects and confidence limits for other levels of treatment efficacy and precision, with and without survivors (Couey and Chew 1986).

Japan, Australia, and New Zealand accept quarantine treatment efficacy at 99.99% (at the 95% confidence level), which is obtained by treating 29,956 insects with no survivors (Couey and Chew 1986). Japan requires a total of 30,000 individuals in three to four trials (Sproul 1976), New Zealand requires three replicates of 10,000 test insects each, and Australia accepts a cumulative total of 30,000 treated insects with no survivors (Heather and Corcoran 1992).

Adjustments may be made to the number of test insects to offset control mortality when control mortality is known (Follett and Neven 2006). Generally, the number of insects tested should be increased to $[n/s]$, where n is the number of insects required for a predetermined level of quarantine security (99.99–99.9968% mortality), and s is the proportion of survivors in the control. For example, if 99.99% mortality at the 95% confidence level is the desired level of quarantine security, and survivorship in the control is 90%, then the number of test insects should be increased from 30,000 to 33,333 insects. Landolt et al. (1994) pointed out that the probit 9 standard may be too stringent for commodities that are rarely infested or are poor hosts, and hence a less severe postharvest treatment might still provide quarantine security. In this case, fewer insects may be needed during research to develop quarantine treatments (Follett and McQuate 2001).

Current Standard and Sample Size. National and regional standards have been developed to harmonize methods used by participating countries in the application of phytosanitary measures. The New Zealand Ministry of Agriculture and Forestry (MAF) has the only published national standard for the determination of fruit fly host status (NZMAF 1994), and the Asian Pacific Plant Protection Commission (APPPC) has prepared a draft of a regional standard with guidelines for the confirmation of nonhost status of fruits and vegetables to tephritid fruit flies (APPPC 2005). To date, an international standard for nonhost status determination is lacking. The New Zealand MAF and APPPC standards are based on methods proposed by Cowley et al. (1992). Several host status determination studies also have followed the methods proposed by Cowley et al. (1992) to demonstrate nonhost status to fruit flies for particular commodities (e.g., Aluja et al. 2004, Mexican 'Hass' avocados).

Cowley et al. (1992) proposed a three-tiered testing protocol and decision tree involving laboratory cage tests with punctured fruit, laboratory cage tests with unpunctured fruit, and field cage tests with unpunctured fruit attached to the tree. The laboratory cage trial with punctured fruit involves exposing 500 g of fruit to a number of gravid females to ensure that 250–500 eggs are laid, replicated five times. In assessing the results, if adults are reared from a single control replicate of a known host fruit exposed to gravid females and no adults are reared from the five replicates of trial fruit, then the trial fruit is declared a nonhost and further testing is unnecessary (Cowley et al. 1992). This means nonhost status could be demonstrated by testing as few as 1,250–2,500 eggs and <100 fruit.

The fruit fly host status testing protocol set forth by Cowley et al. (1992) is a far less rigorous level of testing than has been required to demonstrate the efficacy of traditional quarantine treatments (99.99 or 99.9968% mortality at the 95% confidence level). The level of confidence associated with treating a number of insects with zero survivors is given by the following equation:

$$C = 1 - (1 - p_u)^n \qquad [1]$$

where p_u is the acceptable level of survivorship and n is the number of test insects (Couey and Chew 1986). If we assume that 99.99% mortality is required (p_u = 0.0001), then the level of confidence associated with testing 1,250 insects (as per Cowley et al. 1992) with zero survivors is 11.8% and 2,500 insects with zero survivors is 22.1% (Table 1). This means we have 11.8% and 22.1% confidence that the true survival is <0.0001. Few actual studies have stopped and declared the

Table 1. Confidence level (*C*) produced when various numbers of insects (eggs, larvae, or adults) or fruit (*n*) are used during nonhost testing

No. test subjects (n)	Required proportion mortality $(1 - p_u)$		
	0.999	0.9999	0.999968
625	46.5	6.1	2.0
1,250	71.4	11.8	3.9
2,500	91.8	22.1	7.7
5,000	99.3	39.4	14.8
10,000	99.99	63.2	27.4
20,000		86.5	47.3
30,000		95.0	61.7
93,623		99.99	95.0

Confidence level is calculated as $C = 1 - (1 - p_u)^n$.

commodity a nonhost solely on the basis of a negative result from laboratory cage tests with punctured fruit, but the level of additional laboratory and field cage testing and fruit collection has been variable. Equation 1 can be rearranged to determine the number of insects that are required for testing for a given level of confidence.

$$n = [\log(1 - 0.95)/\log(1 - p_u)] \qquad [2]$$

Equation 2 calculates how many insects or fruit (n) must be tested with no survivors so that we will have 95% confidence (C, as a proportion) that the survival proportion is below a predetermined level (p_u) (Couey and Chew 1986).

Does the Number of Fruit Flies or Fruit Determine Sample Size? Host status testing assumes there may be variation in the fruit fly population for host acceptance and use, or variation in the expression of host plant resistance potentially allowing fruit fly survival. The question arises of whether the sample size used to determine the level of efficacy and confidence should be determined by the number of fruit flies (eggs, larvae, or adults) exposed to fruit, or the number of fruit exposed to fruit flies. An ideal host status study would include laboratory, field cage and natural infestation experiments. In laboratory and field cage host status experiments (e.g., Cowley et al. 1992), gravid adult female flies are typically exposed to the fruit. The number of adult fruit flies and the number of eggs they lay in the fruit can be manipulated. The number of eggs oviposited can be determined by dissecting a subsample of the fruit to count eggs (e.g., Spitler et al. 1984). Later, the number of emerging adults can be compared with the number of eggs to determine the efficacy of host plant resistance. Where egg inoculation directly onto the fruit is used (e.g., Hennessey and Schnell 1995), the number of eggs is counted beforehand. When egg injection into fruit is used (e.g., Laborda et al. 1990), the number of eggs can be estimated by measuring the number of eggs per unit volume injected. Therefore, in laboratory and field cage tests the number of fruit flies and the number of fruit can be quantified, and typically many fruit flies have been exposed to a small number of fruit.

To determine the natural infestation rate, fruit are collected from the field and dissected to count eggs and larvae, or they are held for adult fruit fly emergence. The numbers of adult fruit flies present in the orchard and the number of fruit that are visited by gravid flies during a defined period or phenological fruit stage is usually unknown, because there are no visible signs of infestation or visitation. Therefore, the sampled number of fruits is used to determine the level of confidence. If the natural infestation rate is low, a large number of fruit samples will be required to determine nonhost efficacy with 95% confidence (e.g., Hennessey et al. 1992). Hence, confidence levels for fruit flies and fruit can be calculated for laboratory, field cage, and natural infestation experiments and used together to assess host status.

Host resistance may act on one or more stages of the fruit fly. In nature, adult fruit flies must first be attracted to the host and then the females must be capable of ovipositing successfully (Prokopy and Roitberg 1989). Eggs deposited in the host must hatch, and larvae must successfully feed and develop to maturity. Confidence levels determined from host status testing may be misleading if the sample size is determined for an inappropriate fruit fly life stage. For example, if the host is acceptable for oviposition but a nonhost for larval development (i.e., antibiosis resistance), estimating sample size for eggs or neonates is more appropriate than estimating sample size for adults. Conversely, if the peel poses a significant barrier to oviposition (i.e., morphological nonpreference resistance) into an otherwise acceptable host (e.g., Birke et al. 2006), determining the sample size for the adult stage is most appropriate.

Nonhost Case Studies. Eleven representative studies from the literature were examined to determine the number of adult fruit flies and commodity units used during nonhost testing and illustrate how to calculate the level of confidence (Table 2). Nine of the studies reported laboratory cage or field cage infestation studies, and three reported natural field infestation studies. The total numbers of gravid females used in the cage testing ranged from 45 to 14,770, and the total number of fruit included in tests ranged from 44 to 574,296. The corresponding confidence levels for 99.99% mortality ranged from 0.5 to 77.2% and for 99.9968% mortality from 0.1 to 37.7% for adult flies. For numbers of fruit, confidence levels ranged from 0.4 to 100.0% for 99.99% mortality and from 0.1 to 100.0 for 99.9968% mortality. None of the studies exceeded the 29,950 insects needed to demonstrate 99.99% mortality at the 95% confidence level, and only four studies (Armstrong 1991, Burikam et al. 1992, Hennessey et al. 1992, Yokoyama and Miller 1993) sampled sufficient numbers of fruit to demonstrate 99.99% (and/or 99.9968%) mortality at the 95% confidence level or better. 'Sharwil' avocados were declared nonhosts when attached to the tree for oriental and Mediterranean fruit flies after inspection of >114,000 fruit during two seasons with no observed infestation (Armstrong 1991). Mangosteen was determined to be a nonhost for oriental fruit fly from laboratory inspection of 40,000 fruit collected from orchards throughout Thailand during 2 yr (Burikam et al. 1992). Nonhost

Table 2. Estimated sample size and confidence level of host status determination studies resulting in no successful fruit fly maturation

Fruit	Cultivar	Fruit fly species	No. insects tested[a]	Confidence level[b]		No. fruit tested	Confidence level[b]		Source
				99.99	99.9968		99.99	99.9968	
Avocado	Hass	*Anastrepha ludens*	6,500	47.8	18.8	14,542[b]	76.6	37.2	Aluja et al. 2004
		Anastrepha obliqua	6,500	47.8	18.8	14,542[c]	76.6	37.2	
		Anastrepha serpentina	6,500	47.8	18.8	14,542[c]	76.6	37.2	
		Anastrepha striata	6,500	47.8	18.8	14,542[c]	76.6	37.2	
	Sharwil	*Ceratitis capitata*				114,112	100.0	97.4	Armstrong et al. 1991
		Bactrocera cucurbitae				114,112	100.0	97.4	
		Bactrocera dorsalis	1,500	13.9	4.7	117,783	100.0	97.8	
Banana	Dwarf	*Bactrocera dorsalis*	13,500	74.1	35.1	11,541	68.5	30.9	Armstrong 2001
(green)	Brazilian, Cavendish	*Bactrocera cucurbitae*				9,278	60.5	25.7	
Lime	Tahiti	*Anastrepha suspensa*				102,384	100.0	96.2	Hennessey et al. 1992
Longan	Kohala	*Anastrepha suspensa*	180	1.8	0.6	15,800[2]	79.4	39.7	Gould et al. 1999
Lychee	Mauritius, Brewster	*Anastrepha suspensa*	360	3.5	1.1	18,200[2]	83.8	44.1	Gould et al. 1999
Orange	Valencia	*Anastrepha fraterculus*	205	2.0	0.7	100	1.0	0.3	Aluja et al. 2003
Prune	ns	*Rhagoletis completa*	1,800	16.5	5.6	574,296	100.0	100.0	Yokoyama and Miller 1993
Mamey sapote	Magana, Pantin	*Anastrepha suspensa*	800	7.7	2.5	646	6.3	2.1	Gould and Hallman 2001a
Mangosteen	ns	*Bactrocera dorsalis*	750	7.2	2.4	40,000	98.2	72.2	Burikam et al. 1992
Monstera	ns	*Anastrepha suspensa*	105	1.0	0.3	44	0.4	0.1	Gould and Hallman 2001b

ns, not specified.
[a] Includes gravid females only.
[b] 99.99 and 99.9968 represent required % mortality.
[c] Includes dissected fruit, packinghouse fruit, and fruit on trees caged with fruit flies.

status of 'Tahiti' limes for Caribbean fruit fly, *Anastrepha suspensa* (Loew), was shown after inspection of 102,384 unsorted, ungraded packinghouse fruit from 184 different groves on 60 harvest dates and finding no infested fruit (Hennessey et al. 1992). Fresh prunes were determined to be a nonhost for oriental fruit moth, *Grapholita molesta* (Busck), and walnut husk fly, *Rhagoletis completa* Cresson, after sampling ≈574,296 packinghouse culls (Yokoyama and Miller 1993).

Using large numbers of fruit and insects during nonhost testing is of critical importance when there is some evidence that the insect may develop to the pupal or adult stage in the fruit, however rarely or slowly. For example, two *B. dorsalis* larvae were found in a fallen, partially broken-open mangosteen fruit in an orchard in Rayong Province in Thailand (Burikam et al. 1992); however, no oriental fruit fly eggs or larvae were found in 40,000 mangosteen fruit collected from orchards throughout Thailand, and no eggs were found on harvested fruit placed in cages with 1,000–1,500 adult flies. Aluja et al. (2003) found that *Anastrepha fraterculus* (Wiedemann) rarely oviposits into oranges, *Citrus sinensis* (cv. Valencia), or grapefruit, *C. paradisi* (cv. Ruby Red). When oviposition occurred, larvae did not develop in oranges and rarely developed in grapefruit; when larvae developed and pupated in grapefruit, pupae were of low weight and emerging adults lived only a short time and did not reproduce (Aluja et al. 2003). The study included laboratory and field cage tests that were well designed and well replicated but included a total of only a few hundred fruit and fruit flies, resulting in low confidence (Table 2). Hass avocados in Michoacan, Mexico, were recently reported as a nonhost for several species of *Anastrepha* fruit flies (Aluja et al. 2004). In a 1-yr study, when mature fruit on the tree were exposed to female oviposition in sleeve cage tests, four of 1,300 fruit were infested by Mexican fruit fly, *A. ludens* (Loew), and larvae developed to the pupal stage, but no adults emerged (Aluja et al. 2004). Fruit from orchards (n = 7,936) where *Anastrepha* spp. fruit flies were caught in traps and fruit from packinghouses (n = 1,620) were not infested with *Anastrepha* spp. fruit fly eggs or larvae. Although no flies developed to the adult stage on avocados, the study included fruit collection in the field for only one season and involved a relatively small number of fruit for each fruit fly species (n = 14,542), resulting in only moderate confidence (Table 2). Sample size and confidence levels would be significantly higher if the number of eggs laid by adult flies is estimated and used in calculations. For example, at one of the study sites, Hass avocado fruit were dissected, and it was determined that wild Mexican fruit flies laid an average of 12.1 eggs per clutch and 109 eggs per fruit (Aluja et al. 2004). If Mexican fruit fly deposited 109 eggs in each of the avocado fruit in the forced-infestation test (n = 1300), the estimated sample size would be 141,700 eggs with no survivors to adult, which would exceed the probit 9 response standard (Table 1).

In some cases, conducting large-scale nonhost testing is impractical. The crop may be planted on a limited scale (Gould and Hallman 2001a) or the quarantine pest may be difficult to rear in the laboratory. In these cases, equation 1 can be used to determine the level of confidence associated with the sample size of the nonhost testing that is conducted.

In summary, several studies have demonstrated nonhost status with a high level of efficacy and confidence; therefore, a nonhost protocol could probably be developed as a stand-alone quarantine measure. Other studies have demonstrated nonhost status with only a moderate or low level of confidence, and in these cases additional measures would be justified to ensure quarantine security. Thus, we stress the importance of including a confidence level with efficacy information on nonhost status.

Survivors. Theoretically, probit 9 mortality, if that is the required level of efficacy, can be achieved while allowing for survivors if a sufficient number of insects or fruit are tested, which could pose a dilemma when determining the host status of a commodity. Couey and Chew (1986) provide an equation to estimate the confidence levels for efficacy when only a few insects survive on a host,

$$\sum_{x=0}^{x=s} e^{-m}m^{x}/x! = 1 - C \qquad [3]$$

where m is $n \times p_u$, n is the number of insects or fruit sampled, p_u is the maximum allowable infestation proportion (e.g., 0.0001 for 99.99% mortality), s is the number of survivors, and C is the confidence level. This equation uses the Poisson distribution law and assumes large n and small p_u (Couey and Chew 1986). Spitler et al. (1984) tested the host status of lemons to Mediterranean fruit fly in laboratory cage tests. From an estimated total number of 515,982 eggs deposited in fruit, only five survived to the pupal stage for a successful development rate of 0.0000097. This rate of mortality is better than probit 9; moreover, we have 99.9% (increase from 95%) confidence that mortality is 99.9968%. However, the distribution of survivors during host status testing may be an important consideration when determining the level of host resistance. In the case mentioned above, all the survivors came from just one of the test dates using an estimated 31,800 eggs, for a survival rate of 0.0002. For this sample alone, our confidence that the mortality is 99.9968% is only 0.066%.

Although it is possible to determine the numbers to be tested where one or more survivors occur, few countries will accept a traditional quarantine treatment where there have been failures. Likewise, any development of a fruit fly during host status testing, however rare, suggests that the use of nonhost status alone may not provide an acceptable level of risk. Lemons are imported from Spain by using a nonhost protocol based on the data from Spitler et al. 1984 as discussed above. In 2006, live Mediterranean fruit fly larvae were detected in 'Verna' lemons shipped from Spain. The survivors found during host status testing with 'Eureka' and 'Lisbon' lemons (Spitler et al. 1984) were an indication that the nonhost status protocol for lemons could fail under certain conditions. The presence of survivors during nonhost status testing typically suggests that additional control measures or safeguards are needed to reduce risk to acceptable levels.

Year-to-Year Variation. The physiological basis for host antibiosis or nonpreference to a quarantine pest is often not understood (Greany 1989, 1994). Therefore, establishing nonhost status can be difficult, because researchers must conduct infestability studies under a variety of conditions (e.g., climate, growing region, elevation, early and late season, different orchard management practices) and over multiple years. Also, sufficient numbers of fruit or insects must be included in the study to have high confidence in the data, as discussed above. The importance of year-to-year variation and research methodology was illustrated in the development of a nonhost protocol for Hawaiian Sharwil avocados. In the laboratory, Sharwil avocados with stems attached were not susceptible to fruit fly infestation for up to 12 h after harvest, but then they became good hosts (Armstrong 1991). Inspection of >114,000 harvest mature fruit over two seasons indicated no fruit fly infestations, and the data were used to approve a nonhost status export protocol from Hawaii to Alaska. Another study (Oi and Mau 1989) demonstrated that Sharwil avocados became infested at low levels when Mediterranean fruit flies and oriental fruit flies were caged with fruit still attached to the tree, casting doubt on the nonhost status. In 1992, live larvae were found in fruit samples from orchards and the protocol was suspended. In the first year of a follow-up study, oriental fruit flies were found in 15 of 3,248 harvest-ripe Sharwil avocados collected off the tree, but in the second year 0 of 5,004 fruit were infested (Liquido et al. 1995a). Of the 15 infested fruit, five fruit had no visible indications of infestation, emphasizing the low level of sensitivity of detection of infestation as determined by visual external inspection in mature green avocados. This study also demonstrated that "firm ripe" and "fully ripe" fruit occur on the tree late in the season (2.2%) and are much more likely to be infested than mature green fruit. The mechanism of resistance in avocado against fruit flies was not determined.

When multiple host status determination studies have been conducted for a pest over several years they may be combined to increase the sample size during calculation of the level of quarantine security. For example, >10 million Hass avocados were cut and inspected for the potential presence of fruit fly larvae during a 6-yr period of limited harvest exports from Mexico to the United States without finding any live larvae. This information was analyzed to report likelihood of entry of fruit flies and confidence levels, and it was combined with the results presented in Aluja et al. (2004) to make a decision about the risk of expanding Hass avocado exports to the United States (Federal Register 2004).

Supporting Evidence for Nonhost Status. There are other factors that can affect how nonhost status is evaluated as a measure if the confidence level is low or moderate (e.g., no survivors after testing of low or moderate numbers of insects). In most cases, nonhost testing is done because the commodity has a record of being a host. The historical records and literature on host usage may be used to corroborate or cast uncertainty on the nonhost studies. The records and literature should be critically examined to determine the quality of the evidence. Many published host records give little or no information on the maturity stage, variety, whether the commodity was picked or found on the ground, whether the commodity was grown in a backyard or commercially, whether the commodity was damaged, or other important factors that can affect host status.

Host record databases can facilitate critical review of the literature. For example, annotated host record databases were compiled for Mediterranean fruit fly (Liquido et al. 1991) and *Anastrepha* sp. (Norrbom 2004). They contain information on fruit maturity and condition and type of investigation that was used to determine the host status. Multiple records of a host being infested by a species of fruit fly weighs against considering the nonhost approach for the species, whereas the absence of evidence for a specific host–quarantine pest association may be the result of a recent pest invasion into the range of the host or the fact that the association has not been investigated.

Demonstration of nonhost status with a low or moderate level of confidence does not preclude its use as a measure. Such a case could justify reducing the strength of a postharvest quarantine treatment if both are used in combination and their combined effects provide acceptable quarantine security. A commodity that is infested only rarely could be used in combination with low prevalence of the pest in the growing area or as a component in a systems approach (Follett and Neven 2006). The components of a systems approach can vary widely, but they commonly include pest survey, trapping and sampling, field treatment, cultural practices, postharvest safeguards, limited harvest period, limited sales distribution, and restrictions on crop maturity at harvest, which cumulatively provide quarantine security.

In conclusion, researchers should conduct infestability studies under defined conditions and with sufficient numbers of fruits and insects to convincingly determine nonhost status of a commodity. Quantitative methods are available to determine the level of confidence associated with a particular sample size. For example, if no survivors are found after exposing 30,000 insects to harvest mature fruit or sampling 30,000 fruit from the field, we can say with 95% confidence that resistance is 99.99% effective. When low numbers of insects or fruit are tested, nonhost status may still be used in combination with other measures to provide an acceptable level of quarantine security. Incorporating sample size and confidence levels into host status testing protocols along with efficacy will lead to greater consistency by regulatory decision-makers in interpreting results and, therefore, more technically sound decisions on host status.

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