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DEVELOPMENT OF ATTRACTANTS FOR
MONITORING CARIBBEAN FRUIT FLIES
(DIPTERA: TEPHRITIDAE)

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ABSTRACT

Methods for monitoring Caribbean fruit flies, *Anastrepha suspensa*, are reviewed and areas of essential research required are suggested. Types of attractants discussed include food, pheromone, visual, and acoustical cues. Additionally, current information regarding the chemistry of male-produced pheromones and analytical methods for their detection and elucidation are reported. The predominant monitoring methods used are either cumbersome to deploy or ineffective. However, attempts to develop improved traps are often hindered by the lack of an understanding of the underlying fly response. The linking of currently available analytical techniques with an understanding of *A. suspensa* behavior should allow optimization of attractants and traps, and provide powerful methods for population monitoring.

RESUMEN

Se revisan metodos de monitoreo de la mosca del Caribe *Anastrepha suspensa* y se sugieren areas esenciales para investigaci3n. Los tipos de atrayentes discutidos incluyen alimentos, feromonas, trampas visuales y acusticas. Adicionalmente se reporta informaci3n sobre la qu3mica de feromonas producidas por el macho y los metodos analiticos para su detecci3n y determinaci3n. Los metodos actuales de monitoreo son impracticos o poco efectivos. Sin embargo, los esfuerzos de mejoramiento de estas trampas se ven retrasados por la falta de entendimiento de la respuesta de la mosca. Las tecnicas anal3ticas unidas a los conocimientos sobre comportamiento de la mosca del caribe, facilitar3an unos metodos mas poderosos de monitoreo.

The threat of the Caribbean fruit fly (caribfly), *Anastrepha suspensa* (Loew) (Diptera: Tephritidae), in citrus-growing regions of south Florida is of considerable economic importance. Even a small infestation of flies makes all fruit grown in the area suspect and thus unsalable to many potential domestic and foreign markets without costly post-harvest treatments or establishment of fly-free zones (Riherd 1993, Simpson 1993). Because of this threat and the potential for introduction of the caribfly into other citrus-growing states, much emphasis has been placed on detection of this species.

Several approaches have been used to develop detection methods to monitor populations of pest fruit flies (Economopoulos 1989). The traps currently used for caribflies are either cumbersome to deploy or ineffective, and research in this area is often hampered by a lack of understanding of the fly attraction behavior. It is beyond the scope of this article to provide an in-depth review of all systems tested for caribfly detection. It is, however, germane to the discussion of caribfly attractants to provide a brief explanatory review of the various attractants. We present in this discourse an overview of several attraction methods with discussions regarding research pitfalls and opportunities involved in developing improved lures for caribfly detection.

FOOD ATTRACTANTS

Review

Adult fruit flies require sugar to survive (Christenson & Foote 1960), and honeydew is recognized as an important food source for adult tephritids (Hagen 1958). Females also require protein to ensure fecundity, and the protein requirement is the primary basis for traps for detection of *Anastrepha* spp. (Anonymous 1989). A number of hydrolyzed proteins have been used as baits to attract fruit flies, but comparisons of several proteins indicated that hydrolyzed torula yeast (HTY) was superior for attracting caribflies (Lopez et al. 1971, Burditt 1982). Addition of sodium borate (borax) to the HTY reduced bait decomposition without affecting catch (Lopez & Becerral 1967), and a pelleted formulation of HTY and borax was developed to facilitate field placement of baits (Lopez et al. 1968). McPhail traps, bell-shaped glass traps with a water reservoir (Newell 1936), baited with 5-6 HTY-borax pellets are currently used for monitoring caribfly populations in Florida (Anonymous 1989). Other baits and/or trap types have been tested for caribfly detection. Non-baited yellow disks were inferior to HTY-borax-baited McPhail traps (Witherell 1982), but yellow sticky board traps with ammonium acetate or 3-phenylpropyl-2-methylpropanoate were as effective as HTY-borax baited

McPhail traps (Burditt et al. 1983). Davis et al. (1984) compared baited and non-baited triangular cardboard traps (Jackson traps) to HTY-borax baited McPhail traps. Addition of bait (cotton wicks soaked in aqueous yeast hydrolysate) to Jackson traps increased capture of females, but not males. However, a greater number of flies and a higher proportion of immature female flies were caught by the baited McPhail traps. In studies in a guava grove in Homestead, FL, yeast hydrolysate-baited Jackson traps and HTY-borax-baited McPhail traps were equally effective when population densities were high (in the fall), but the baited McPhail traps were superior in the spring when caribfly population densities were low (Mason and Baranowski 1989).

Bacteria on plant surfaces may serve as a protein source for adult tephritids in nature (Drew et al. 1983). Addition of actively growing bacteria to protein suspensions increased attraction of the Queensland fruit fly, *Dacus tryoni* Froggatt, over protein suspensions without bacteria (Drew & Lloyd 1989). Bacterial by-products may similarly attract caribflies. McPhail traps baited with a fermenting mixture of citrus juice and brown sugar were successfully used to trap caribflies in early studies in Florida (Newell 1936). Davis et al. (1984) speculated that the increased attraction by HTY-borax in McPhail traps versus aqueous yeast hydrolysate-soaked cotton wicks in Jackson traps was due to volatiles from breakdown products of the HTY in solution.

Research Opportunities

Although a number of protein-rich materials have been screened for attractant activity, there is little information on the chemicals responsible for caribfly attraction. Chemical constituents of various protein attractants used for fruit flies have been investigated (Morton & Bateman 1981, Buttery et al. 1983), but the attractiveness of individual constituents was not examined. The development of a protocol for interfacing the behavioral response with food chemical isolates is necessary to determine the specific volatiles responsible for caribfly attraction. This should lead to the development of less cumbersome traps baited with the attractant in a controlled-release formulation. The results of this research would circumvent difficulties involved in use of currently available food-based traps.

VISUAL ATTRACTANTS

Review

Fruit flies may use a number of visual cues to locate hosts, and visual cues alone or in conjunction with chemical cues have been used successfully in developing traps for fruit flies (Prokopy 1975, Landolt et al. 1988). Greany et al. (1977, 1978) found that caribflies were strongly attracted to, and could be trapped by fluorescent orange sticky traps that reflected maximally at 590 nm (Arc Yellow, Day-Glo Color Corp., Cleveland, Ohio). The trap catch was predominantly female, and most of the trapped females were sexually mature. Thus, the observed attraction was the result of fruit-seeking rather than foliage-seeking behavior (Greany et al. 1978). Davis et al. (1984) explored a number of trap designs and found that Jackson traps with orange stripes captured more caribflies than solid orange or white traps. Sivinski (1990) found that 20-cm diam. orange spheres were significantly more effective at catching male caribflies than smaller or differently colored spheres, but female caribflies were trapped equally on 20-cm green spheres.

Research Opportunities

Caribflies are responsive to visual cues and considerable research has focused on shapes and colors attractive to caribflies in hopes of developing an effective dry trap alternative to liquid-baited McPhail traps. However, dry trap use is currently restricted because of the need for sticky material on the trap surface. In addition, significant numbers of other insects are attracted to these traps and considerable effort is required to sort unwanted insects from the captured caribflies. Incorporation of chemical attractants that attract only caribflies may enhance the utility of these traps if the number of non-target insects could be reduced. Research related to the development of better sticky material that could be regenerated in the field is also needed.

ACOUSTICAL ATTRACTANTS

Review

In concert with the release of pheromone *vide infra*, males produce an audible signal, i.e. call. Production of male-specific sounds in caribflies was documented by Webb et al. (1976). Calling males produced a pulsed and audible signal but fell silent as a female approached. If the female was receptive and made contact with the male, the male produced a precopulatory song. If the female was unreceptive, the male resumed calling. Females may use these sounds to assess male size and vigor. Larger males called earlier in the sexual activity period and their calls had shorter pulse-train intervals (Burk & Webb 1983). Male calling increased flight activity of virgin but not mature females (Webb et al. 1983). Attraction of virgin females to male calling sounds has been demonstrated in field-cage tests. Significantly more females were attracted to tape-recorded male sounds than to silent control traps (Webb et al. 1983).

Research Opportunities

The general utility of using acoustical attractants at this time is unknown. Methods for providing these signals in the field at a reasonable cost would be needed. Considering current technology, however, there may be some future role for caribfly acoustical attractants.

PHEROMONE ATTRACTANTS

Review

Potentially the most powerful attractant, and seemingly the most difficult to study, is the male-produced pheromone. Research that began in 1972 and continues to date has not yet resulted in a pheromone-based trap for the caribfly. Early work described courtship and mating behavior of caribflies and documented pheromone production by puffing males (Nation 1972). Female flies were attracted (in laboratory bioassays) to live males, to whole body extracts of males, and to papers that had been placed in cages with puffing males. Subsequent studies by Perdomo (1974) showed that both females and males were attracted to live males, and that male-baited traps caught five to ten times more flies than food-baited traps in field trials using laboratory-reared, virgin flies.

Initial studies on the chemical nature of the pheromone extracted from abdomens of sexually-mature male caribflies resulted in the identification of (*Z*)-3-nonenol and (*Z,Z*)-3,6-nonadienol (Nation 1975, 1977, 1983). Subsequent investigations of abdominal extracts of male flies performed by Battiste et al. (1983) resulted in the identification of two additional components, the lactones, anastrephin (*trans*-hexahydro-*trans*-4,7 α -dimethyl-4-vinyl-2-(3H)-benzofuranone) and epi-anastrephin (*trans*-hexahydro-*cis*-4,7 α -dimethyl-4-vinyl-2-(3H)-benzofuranone). Laboratory bioassays of these compounds showed that all were individually attractive to females, but a blend of all four components was the most attractive to females (Nation 1975). A synthetic mix of the four components, however, failed to attract flies in field trials (Nation 1989). A fifth component, a macrolide (*E,E*)-4,8-dimethyl-3,8-decadien-10-olide) was identified and synthesized by Chuman et al. (1988) and has been named suspensolide. Additionally, β -bisabolene and ocimene have been reported as volatiles emitted by the caribfly (Tumlinson 1988, Nation 1991, Rocca et al 1992). More recently, *E,E*- α -farnesene has been reported (Rocca et al. 1992).

Although significant research efforts have gone into the identification and synthesis of the putative pheromone chemicals released by male caribflies, one is left with the question "So what?" Admittedly, the chemistry of the male caribfly pheromone is complex. A typical chromatogram of volatiles collected from "calling" males contains numerous compounds (Figure 1). Analysis of some of the pheromonal components is further complicated by their thermal lability. For example, the identification of suspensolide was not completed until 1988 and the identification of *E,E*- α -farnesene not until 1992. Elucidation of these components in earlier research was hampered by the lack of appropriate analytical methodologies now available to researchers. Careful consideration must be given to the analyses of compounds such as suspensolide. From studies conducted in our laboratory using synthetic material we have determined two factors that may result in the loss of suspensolide. A loss of approximately 25% of suspensolide occurs when Tenax is used as the adsorbent material (Figure 2). More importantly, the elevated injector temperatures commonly used with split-splitless injectors can result in an additional loss of approximately 75% of suspensolide (Figure 3). Thus, prior to 1988, the method of collection coupled with method of injection resulted in the inability to detect suspensolide as a major volatile of the male produced caribfly pheromone.

To illustrate the various aspects of the analytical protocol currently used in our laboratory for the analyses of male produced pheromone from caribflies, we provide the following brief review of the methods. Although the analytical methods described are used for caribfly research, these techniques, which have been developed over a period of several years, are applicable to identification of other pheromonal systems.

Collection of Volatiles.

A complete description of the system used to in our laboratory to collect volatiles has been recently published (Heath and Manukian 1992). The system (Figure 4) consists of three parts: 1) a plastic coupler used to press charcoal filters against the glass flange of the insect holding chamber, thus purifying inlet air; 2) a glass insect holding chamber constructed of Pyrex glass with a flange and 50/55 male ground-glass joint outlet; and 3) a multiport collector base. Manual switching for collection of volatiles on one of the three collector traps or purge is accomplished using a four-way, multiport valve. A flowmeter controls the amount of air pulled by a vacuum pump through the system. An aluminum screen, placed upwind of the collector traps in the insect holding section, keeps the insects upwind of the collectors. Volatiles are collected on traps using Super-

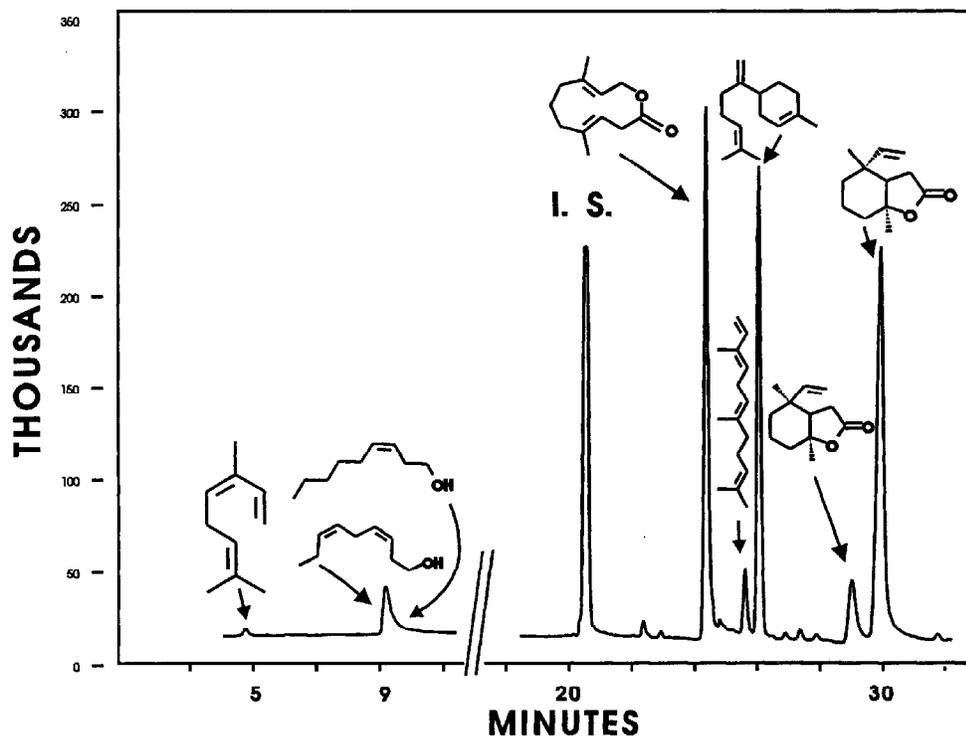


Fig. 1. Gas chromatogram of pheromonal components collected from male caribflies. Column used was 50 m x 0.25 mm ID BP-1® (apolar phase).

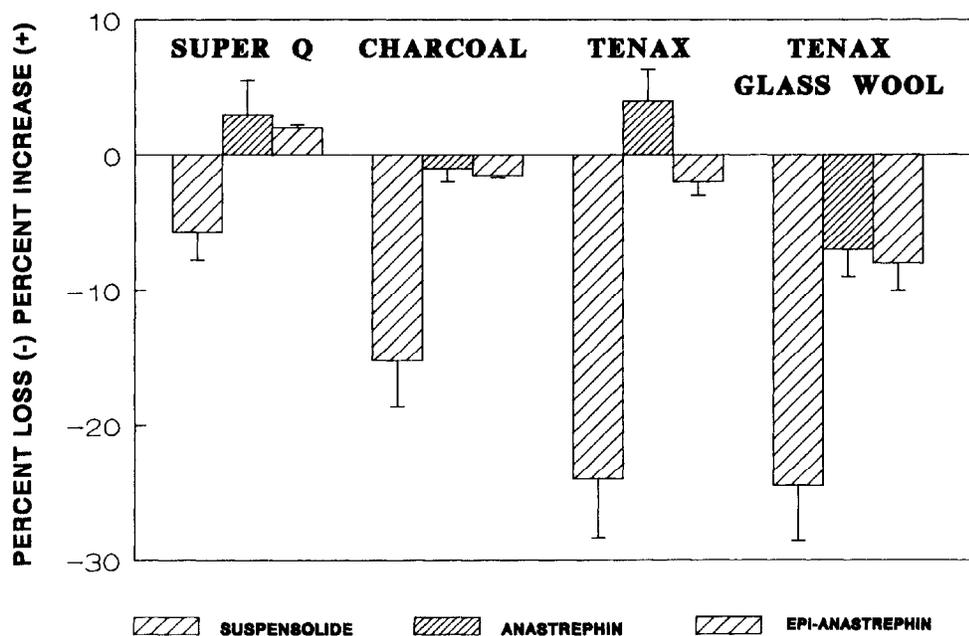


Fig. 2. Average percent loss of suspensolide and rearrangement products that occur with different type of adsorbents used in caribfly pheromone collection (n=3). Error bars indicate standard deviation.

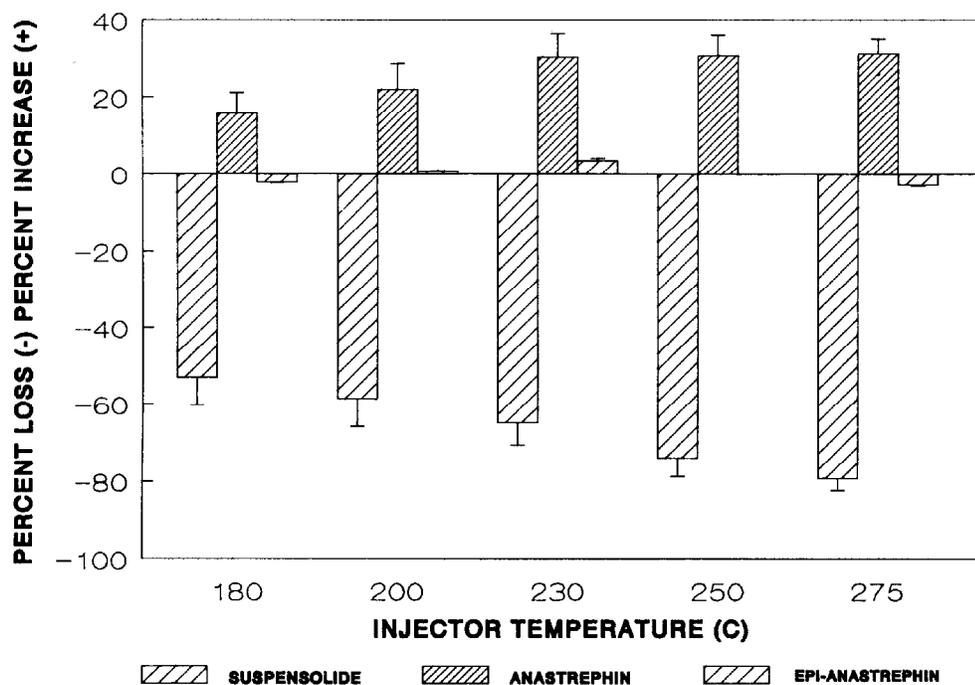


Fig. 3. Average percent loss of suspensolide and rearrangement products that occur with different injector temperatures when split-splitless injection is used in caribfly pheromone analyses ($n=3$). Error bars indicate standard deviation.

Q® (Alltech Assoc. Inc., Deerfield, IL) as the adsorbent and then eluted with methylene chloride. An internal standard is added for subsequent analyses.

Analysis of Volatiles.

Capillary gas chromatography is conducted using a retention gap column prior to the analytical capillary column. This system permits the injection of 5-100 μ l of solvent and collected volatiles without concentration and without loss of performance of the analytical column. A combination of three fused silica columns connected in series using GlasSeal® connectors (Supelco, Inc. Bellefonte, PA) is used. The primary fused silica column (8 cm x 0.5 mm ID) is connected to the injector and the retention gap column. This primary column permits the use of 0.4 mm O.D. stainless steel needles with a septum injector. Selection of retention gap columns for use with the analytical column is determined by the phase ratio of both columns (Grob 1982; Murphy 1989). For the purpose of caribfly pheromone analyses, the retention gap columns used are 10m x 0.25 mm ID trimethyl silane deactivated fused silica (Quadrex, New Haven, CT). The analytical column typically employed for analyses is a 50 m x 0.25 mm ID BP-1® (apolar phase).

Gas chromatographic analyses are conducted using a Hewlett-Packard Model 5890 gas chromatograph, equipped with a cool-on column capillary injector (septum injector) which is held at ambient room temperature during injection of the extracted pheromone sample. Detection of the compounds is performed by a flame ionization detector. Helium is used as the carrier gas at a linear flow of 18 cm/sec. The following temperature program is used — the column is held isothermal at 60°C for 2 min and subsequently the oven is temperature programmed at 20°/min to 160°C. Chromatographic data is stored and analyzed in a Nelson 4000® data system.

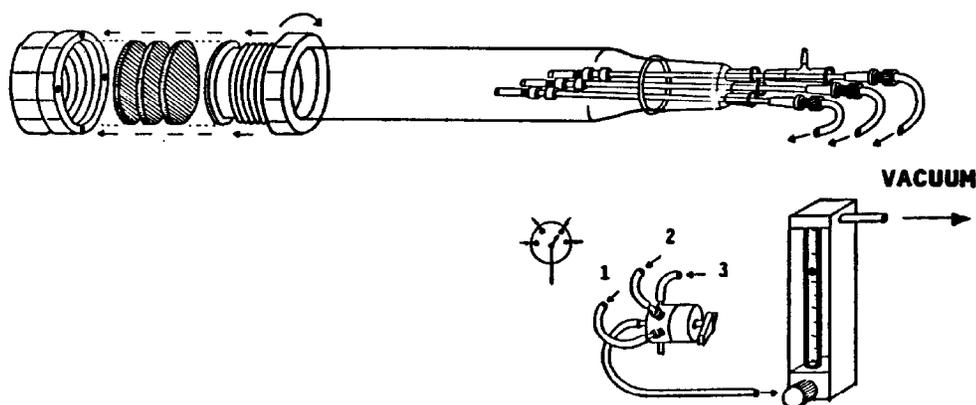


Fig. 4. Illustration of collection system used to collect pheromone emitted by Caribbean fruit flies.

Confirmation of known pheromonal compounds and identity of new compounds is performed using a combination of spectroscopic methods. Initial structure identification is attempted using mass spectrometry. In our laboratory, a gas chromatograph with a cool-on column capillary injector is coupled to a Finnigan Ion Trap[®] mass spectrometer. We obtain both electron impact (EI-ITDMS) and chemical ionization (CI-ITDMS) spectra of the compounds(s). Typically the reagent gas used for CI-ITDS is isobutane. Occasionally, when additional information is desired, CI-ITDS is obtained using ammonia or methane as the reagent gas. Often the identity of new structures require additional spectroscopic information for their delineation. Infrared spectra of compounds can be obtained using vapor phase and solution infrared spectra and are obtained in our laboratory using a Nicolet 20SXC[®] GC-FTIR spectrometer. Samples for vapor phase spectra are introduced to the FTIR spectrometer via a gas chromatograph equipped with cool-on column capillary injector. If the structure is still uncertain, proton nuclear magnetic resonance spectra are obtained [¹H] with a Bruker AC300 (Bellica, MA) 300 MHz Fourier transform spectrometer using either CDCl₃ or C₆D₆ as the solvent and tetramethyl silane as an internal reference. Additional information (although somewhat dated) regarding the spectroscopic methods can be obtained in a review article by Heath and Tumlinson (1984).

Research Opportunities

Analytical methods for the detection and elucidation of the pheromonal components of the caribfly are available and have been extensively researched. Although some bioassay systems have been reported for determining female caribfly response to male-produced pheromone, there is a general lack of knowledge of the behavior associated with the response. What is required is a discriminating attraction bioassay that could be used to provide information relating to relevant response criteria of the pheromonal components. Subsequent to the development of a rigorous bioassay, the evaluation of a pheromone-based trapping system for caribfly could be undertaken using synthetic pheromone compounds which have been formulated to release the chemicals at a rate and ratio used in nature. Further optimization may provide a powerful attractant for female caribflies. Such a system would have a significant impact not only for use as a monitoring tool, but also have potential as a female caribfly annihilation system.

TRAP EFFICIENCY

Trap efficiency is influenced by fruit fly behavior and reflects the physiological state of the fly. Food-baited traps tend to catch more females than males due to the greater food demands of reproductive females as well as the tendency for males to feed in the territory they are defending (Dodson 1982). On guava, females use immature green fruit for oviposition and mature pale yellow fruit for feeding (Burk 1983). Thus, female attraction to green versus orange substrates should reflect physiological condition. Preferential male attraction to orange versus green spheres (Sivinski 1990) may reflect either food-seeking or mate-seeking behavior. Dodson (1982) found that laboratory-reared females spent more time on fruit, either engaged in feeding or oviposition activity, while males spent little time on fruit and obtained most of their food from the leaf surface. In contrast, Burk (1983) observed wild flies, and frequently found males on fruit, either feeding or courting females. When not on fruit, males spent most of their time on leaves either defending territory from other males or advertising for females (Dodson 1982).

Traps combining several attractant cues have had mixed success. Painting all or part of an HTY-borax-baited McPhail trap orange color failed to increase catch (Burditt 1982). Male-baited 20-cm diam orange spheres, however, caught significantly more males and females than food-baited spheres or McPhail traps (Sivinski 1990). The combination of sound and pheromone (whole body extract) did not increase catch over pheromone alone, and traps with sound and/or pheromone were not as attractive as traps with live males (Webb et al. 1983).

Calkins et al. (1984) determined the probability of detecting various populations of caribflies with HTY-borax-baited McPhail traps. Recovery rates were low, with 11-15% of flies recovered from field-released populations of 90-999 laboratory-reared flies per 0.4 ha. They projected that ≥ 33 traps per 0.4 ha were needed to detect low populations of 9 flies per 0.4 ha (detection probability = 90%). Fewer traps, however, were needed to detect or monitor higher populations. An intense trapping program, used to delineate new fruit fly infestations, places 80-40-20-10-5 traps per square mile in a grid covering 81 square miles (Anonymous 1989). Monitoring known populations requires a lower density of 5-10 traps per square mile (C. O. Calkins, personal communication).

Research Opportunities

It is apparent from the review of food-based attractants that a greater understanding of the physiology of the caribfly as it relates to attraction to various cues would facilitate the use of such attractants. Further research is needed to understand the relationships that exist between nutrients needed by adult flies and the volatile cues produced from natural sources. This should lead to development of efficient trapping systems to capture flies attracted to these lures.

SUMMARY

The success in developing better attractants for the caribfly may require significant changes in the directions that are currently being used to achieve this goal. Considerable effort must be placed in obtaining a better interface between the understanding of the biology of the insect and the attractiveness of the semiochemical being investigated. Continued isolation and identification of chemicals at a rapid pace without determining their potential usefulness in the field will most likely provide only marginal improvement

in caribfly attractants. An integrated approach in which the insect response to the semiochemical is demonstrated and understood should provide a significant series of excellent lures for detection and perhaps even control of caribflies.

ACKNOWLEDGEMENTS

We wish to thank A. Manukian and B. Dueben for technical assistance and discussions.

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