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PROEXAG II

COMPONENTE AGRICOLA DEL PROYECTO DE APOYO TECNOLOGICO PARA LAS INDUSTRIAS DE EXPORTACION DE CENTROAMERICA Y PANAMA

REGIONAL WHITEFLY WORKSHOP
Managua, Nicaragua

Assignment Number: ST-180

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DATE: November 15, 1993

TO: Chemonics International
Atn. Dr. Dale Krigsvold
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FROM: Guy L. Bush

SUBJECT: Report on short-term assignment to ST-180/Whitefly Workshop/ Managua, Nicaragua

Upon my arrival in Managua on October 19, 1993 I consulted with one of the workshop organizers, Dr. Pamela Anderson and several of the invited speakers on aspects of the origin, distribution and taxonomic status of different strains of the white fly, *Bemisia tabaci*.

On the following day, Oct. 20, I presented my talk entitled "Biotypos de Insectos" which focused on the problems surrounding the origin and identification of host races and cryptic species. I emphasized the fact that the use of the term 'biotype' was misleading as many different kinds of taxa masquerade under this term ranging from forms that represent non-genetic polyphenisms, genetic polymorphisms, geographic races, host races to distinct species. I presented examples of each category and explained how to establish the status of host associated populations. Distinctions between the various levels of differentiation are essential for the development and implementation of a successful control program. I spent some time explaining how the status of host races and cryptic, morphologically indistinguishable species on the apple maggot fly, *Rhagoletis pomonella*, and its relatives had been established and how this knowledge influenced the management of this pest over the years. I then briefly reviewed the recent research on the white fly and discussed how available information indicated that the new destructive "biotype" causing widespread problems throughout Central America and southwestern USA represented a distinct species that had been introduced, possibly from the Old World tropics. I emphasized that an in-depth molecular based phylogenetic study, which included all known species and 'biotypes' of *Bemisia* ,was required before the origin and taxonomic relationships of the various populations could be established. I indicated several important unresolved problems concerning the various 'biotypes' that required further study. such as; 1) the genetic basis for the esterase phenotypes (mono- or polygenic, X-chromosome linkage); 2) the incidence of parthenogenesis; 3) the origin of the poinsettia or *B*-biotype (= species); 4) the basis of inter-population incompatibility (mate recognition, genomic or cytoplasmic incompatibility or combinations thereof).

During the reaming sessions of the workshop and in the evenings I consulted with workshop participants and found the exchange of information very helpful and stimulating. I believe with further research the status of the various populations of *Bemisia tabaci* can be resolved and programs implemented to limit the dispersal of this pest to other areas of the world and reduce the introduction of other related species and host races.

I returned to Okemos, MI on October, 24, 1993.

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PRESENTATION OF GUY BUSH

MANAGUA WHITEFLY WORKSHOP

Wednesday, October 20, 1993

Several kinds of taxa masquerade under the term biotype as illustrated in slide # 1 (see Diehl & Bush, 1984 for examples and details).

SLIDE 1 *"BIOTYPES"*

1. Non-genetic polyphenisms
2. Polymorphic or polygenic variation within populations
3. Geographic races
4. Habitat (host) races
5. Species

I will briefly define these terms and provide an example of each.

SLIDE 2 *POLYPHENISM*
(Phenocopies or Ecomorphs)

Identical genotype produces different phenotypes under different environmental conditions.

Examples: "Seasonal or environmental polyphenism" Solitary and gregarious "phases" of several migratory locusts. Differ in color, morphology and behavior. Difference continuous, inducible, and partially reversible in a single individual. Caused primarily by crowding and change in food quality.

Also common in many groups like aphids. Early season forms are wingless (apterous) and reproduce parthenogenetically. Late season forms are winged (alate) and reproduce sexually. Polyphenism induced by changes in temperature, photoperiodism and host plant species or quality.

"Host induced polyphenism" encountered in some scale insects and aphids which show marked morphological differences when reared on different or alternative hosts plants.

"Learning induced polyphenism" has been demonstrated in several insects, including the apple maggot fly, *Rhagoletis pomonella*, about which I will say more later.

SLIDE 3 *GENETIC POLYMORPHISM*

Discontinuous phenotypes among individuals within a freely interbreeding population that are the result of allelic variation at a frequency higher than can be maintained by recurrent mutation

"Host plant specific polymorphism" may be caused by single gene polymorphisms. The Hessian fly , *Mayetiola destructor*, is composed of populations each of which can only infest one or two cultivars of wheat. A gene-for-gene relationship between each Hessian fly "biotype" virulence and its preferred susceptible wheat cultivar. Other examples reported in aphids, scale insects, gall wasps. Insecticide resistance polymorphisms are also well studied (which is similar to the kind of resistance Hessian flies display against cultivar antibiosis) and often involve simple genetic polymorphisms. Many other examples involving morphological polymorphisms controlled by a single gene or "super gene" (several tightly linked genes) are well documented such as those involved in mimicry and industrial melanism.

SLIDE 4 *HABITAT RACE*
(Ecotype, Ecological Race, Host Race, Biological race)

A population of a species that is partially reproductively isolated from other conspecific populations as a direct consequence of adaptation to a specific habitat

Several good examples of host and habitat races have been identified among Arthropods such as aphids, flies, moths and butterflies, parasitic copepods, and mites (Bush, 1993b), I will later present details of my own work on the apple maggot fly which has established genetically distinct host races within the last 150 years.

SLIDE 5 *BIOLOGICAL RACE*
(Mayr, 1963)

Non-interbreeding sympatric populations that differ in biological characteristics but not, or scarcely, in morphology .. prevented from interbreeding by a preference for different food plants or other hosts.

This is a widely referenced host race definition which is actually a species definition proposed by Mayr (Mayr, 1963). Mayr apparently does not recognize host preference among species which mate on their hosts as one of the many ecological factors contributing to reproductive isolation. Many arthropods parasitizing animals and plants restrict mating to their hosts. As I will show, host preference is often a sufficiently strong reproductive isolating mechanism to promote and maintain the establishment of sympatric host races.

SLIDE 6 *GEOGRAPHIC RACES*
(Semispecies or Subspecies)

Geographically isolated genetically distinct populations which may or may not be sufficiently different to ensure reproductive isolation if the races become sympatric

The biological status of genetically and/or morphologically distinct geographically isolated populations are the most difficult, often impossible to resolve. Are they insufficiently differentiated and would freely interbreed if sympatric? Are they host races that could maintain their distinct host related genetic and morphological traits yet still be capable of gene exchange? Or have they passed the genetic threshold beyond which the populations would continue to diverge even in the face of gene flow? Just what constitutes such a threshold has yet to be established for any speciation event.

SLIDE 7 *SPECIES*

Natural populations sufficiently reproductively isolated from one another to follow their own distinct independent evolutionary paths.

This definition, which is modified from the biological species concept, was first proposed by the entomologist Benjamin Walsh in 1864 (Walsh, 1864), promoted by Mayr's (Mayr, 1963) . It is conceptually simple, but almost impossible to apply except to those populations that overlap and have the opportunity to interbreed (Templeton, 1987). In fact it is clear that using reproductive isolation as a criteria for awarding species status does not reflect the true nature of what actually keeps populations from fusing if and when they establish contact. Several examples are known in which populations maintain all their "species" characteristics even in the face of gene flow (Howard, 1992).

SLIDE 8 *ESTABLISHING THE STATUS OF HOST-ASSOCIATED POPULATIONS*

Polyphagous host races and host-associated sibling species should be distinguished on the bases of their degree of reproductive isolation and restriction of gene flow under sympatric conditions.

When populations are not sympatric then cross-matings should be carried out under conditions that approach those encountered by the populations in nature. Sterility or reduced viability indicates the populations represent distinct species. When the crosses result in fertile offspring, however, it is seldom clear that the populations would interbreed in nature, particularly if they are associated with different hosts. If crosses can not be made then the status of isolated populations must remain tentative because distinguishing between host races and species is difficult even under ideal circumstances. Until biological and genetic criteria is established for pinpointing the threshold between race and species, only subjective inference on the status of the populations in question can be made.

Separating species from host races is not only of academic interest, but is of practical necessity as well. Effective, long term control strategies may rest on whether or not to treat a pest infesting one host or all host associated populations. Each host race may represent a potential source of individuals genetically preadapted to recolonize a pest free economically important plant. Unfortunately, as you will see, this problem is not easily resolved.

To identify host races the following criteria can be used (Bush, 1993a; Jaenike, 1981):

SLIDE 9 HOST RACES REQUIRE:

1. **Individuals of different host-associated populations must be sympatric and in breeding condition.**

2. **Statistically significant genetic differences exist between sympatric populations over several generations.**

3. **Males and females exhibit genetic variation in host preference that results in host associated assortative mating.**

4. **Males and females show host-associated tradeoffs in fitness.**

5. **No evidence of post-mating reproductive incompatibility. Hybrid incompatibility indicates sibling species not host races.**

Example of host race formation in the apple maggot fly, *Rhagoletis pomonella*.(Bush, 1993a).

SLIDE 10 *RHAGOLETIS POMONELLA MALE ON APPLE FRUIT*

SLIDE 11 *RHAGOLETIS N. SP. MALE ON DOGWOOD FRUIT*

These two sympatric (they share the same geographic area) species are morphologically indistinguishable in all life history stages. They are also interfertile and their hybrids are viable and fecund. They differ in host preference and are fixed for different alleles at several loci. This proves that in nature they do not interbreed. If they did hybridize they would share the same alleles.

SLIDE 12 HISTORY OF HOST SHIFT AND RANGE EXPANSION

In 1862*R. pomonella* was found infesting apples in a commercial orchard in the Hudson River Valley of New York. Soon after this time it was discovered infesting other orchard in the surrounding states and within 50 years it had become a major pest of apples over the entire northeastern part of North America. However, it failed to penetrate into apple growing regions of the southern United States. In Mexico this fly has also independently established a population on apple from the native hawthorn race (Bush, Feder, Berlocher, McPheron, Smith, & Chilcote, 1989).

SLIDE 13 LIFE HISTORY OVERVIEW

Adults emerge in summer after spending the winter in diapause in the ground. After feeding for 10-14 days mating and oviposition occurs on the host fruit. Larvae take about 15-20 days to complete development (Boller & Prokopy, 1976).

SLIDE 14 BEHAVIOR OVERVIEW

Mature males and females are sttracted to host trees by visual and odor cues. Once on the tree both sexes begin searching for dark colored shperes and the odor of ripening fruit. At least 5 esters are involved in host fruit discrimination. Once on the fruit females inspect the surface for marking pheromone that may have been deposited my previous egg-laying females. This pheromone inhibits oviposition. Males search for females and for the presecnce of male arresting pheromone deposited by ovipositing females. Males will patrol and defend a fruit against other males on which a female has oviposited. They also court and attempt to mate with any female that arrives on the fruit (Boller & Prokopy, 1976; Bush, 1993a).

SLIDE 15 ALLOZYMES OF *R. POMONELLA* HOST RACES

The genetic variation in 25 allozyme loci coding for soluble proteins seen in slide 15 were evaluated at many sites throughout northeastern USA where apple and hawthorn grew closely together. Of the 25, six (DIA-2, AAT-2, ME, ACON-2, MPI and HAD) showed highly significant gene frequency differences between the races.

SLIDE 16 GENETIC DIFFERENCES BETWEEN RACES AT GRANT, MI

At Grant, MI samples have been made over a period of 5 years from the same three haw trees and three apple trees. The gene frequency differences in the six loci over this period is represented in slide 16. Samples were made from both larvae and adults aspirated from the tree or reared from fruit under a variety of conditions. Frequency differences were maintained over the sampling period indicating reduced gene flow between the races (Feder, Chilcote, & Bush, 1988).

SLIDE 17 SAMPLING TRANSECTS

Samples were also taken from sympatric populations over east-west and north-south (slide 17) transects (Feder & Bush, 1989; Feder, Chilcote, & Bush, 1989). As at the Grant, MI site, the east to west transects showed consistent differences between the races throughout the sample region. This was not true for the north south transects. In these transects gene frequencies were tightly correlated with local temperatures. The isotherms you see in the slide represent the degree growing day isotherms over the sampling area. Results of the transect are seen in the next slide.

SLIDE 18 GEOGRAPHIC VARIATION BETWEEN RACES

In slide 18 we see the results of the north-south transects made in north-central USA. Here we see a decline in allele frequency differences from north to south at all six loci. These loci appear to be under strong selection and are responding to temperature differences encountered at the local level (Feder & Bush, 1991; Feder, Chilcote, & Bush, 1990). Because these differences between the races are maintained in all stages of development, it is clear that the adults are restricting their mating and oviposition to their prefered host. The results of just ccompleted mark-relerase-recapture study by Jeff Fedder supports this conclusion and also indicates that host preference in these flies is under genetic control (Feder, Opp, Wlazlo, Spisak, Reynolds, & Go, In Prepa).

SLIDE 19 SUMMARY OF DIFFERENCES BETWEEN HOST RACES

1. **Host preference** (Feder, et al., In Prepa)
2. **Eclosion time ~2 weeks apart** (Smith, 1988b)
3. **Rate of maturation (mating period ~3 weeks apart)** (Feder, et al., In Prepa)
4. **Response to host odor**(Frey, Bierbaum, & Bush, 1992; Frey & Bush, 1990)
5. **Rate of parasitism (5% apple, 45% haw) and number of parasitoid species (1 on apple, 2 on haw)** (Feder, Reynolds, & Go, In Prepb)
6. **Ability to learn to recognize alternate host fruit** (Prokopy, Avrill, S. S, & Roitberg, 1982; Prokopy, Cooley, & Opp, 1989)

Based on extensive mark-release-recapture studies over a three year period, the level of gene flow between the apple and haw races each generation has been estimated to be about 6%. The tradeoff in fitness comes primarily from selection acting on eclosion time which is under genetic control, and the benefit the apple race derives by escaping heavy parasitism. If adults eclose too early and infest early ripening apples they may fail to diapause and emerge late the same summer too late to find fruit for oviposition or to complete development before frost. The haw race must time eclosion and maturation to coincide with a narrow window when haw fruits are in suitable condition for oviposition and before it is too late to complete development. Apple flies escape heavy parasitism which is a selective advantage which may compensate for the loss in fitness they may experience feeding on the new host.

Conclusion: The apple and hawthorn populations of *R. pomonella* meet all host race criteria. Whether they already have passed the critical genetic threshold for speciation i.e. the point where they will continue on separate evolutionary paths until all gene flow is terminated can not as yet be

determined. However, *R. pomonella* has been introduced, possibly within the last 20 years, into western North America. In California, Oregon and Washington it infests haws and in some places apple. In Utah sit infests haw and cherry, but not apple, while in Colorado it infests only haw, leaving the apple and cherry uninfested. The reason for its host preferences in these regions is unknown. The difference suggest that these host races are somewhat labile and have their future is unpredictable.

The*Bemisia tabaci* Problem: So what is the status of the many "biotypes" reported for *Bemisia tabaci*? Based on the generally accepted criteria discussed above I reach the following*tentative* conclusions drawing on the compelling biological evidence reported by (Brown, In Press; Perring, Cooper, Rodriguez, Farrar, & Tom S. Bellows, 1993a; Perring, Farrar, Cooper, Tom S. Bellows, & Rodriguez, 1993b) and from a review the literature on this species published since 1987.

SLIDE 20 TENTATIVE CONCLUSION

1. The *A* and *B* "biotypes" in the USA are distinct, non-interbreeding species because:
    a. They have fixed allelic difference at 3 loci
    b. 7 PCR amplified RAPD primers differ greatly
    b. B "Biotype" rapidly replaced A "Biotype"
    c. No heterozygotes found in sympatric populations
    d. Single pair interbiotype matings have failed
2. Many geographically isolated populations of *Bemisia tabaci* represent complexes of host races or cryptic sibling species

Conclusions on the Status of *Bemisia tabaci* Biotypes: It looks like each biotype has its own narrow to broad host range which evolved independently over time throughout the middle latitudes in geographically isolated populations. Host range of each "biotype" depended on the array of potential host plants. Each one of these biotypes may represent either a

complex of host races or cryptic sibling species. With the advent of rapid transportation and an increase in commercial traffic in ornamentals and other plants these geographically isolated populations have been indiscriminately introduced throughout the middle latitudes. On the basis of genetic and biological data these "biotypes" have all the qualities of distinct species. When the B-biotype was introduced to different regions of North America it quickly replaced the resident A-biotype. There is no evidence of interbreeding between these two taxa. If hybridization was occurring then heterozygous individuals bearing traits of both would be encountered in nature. This is not the case. Also, attempts at single pair matings have failed so it appears that many of these geographically isolated populations of *Bemisia tabaci* are might actually be distinct species.

SLIDE 21 STATUS OF OTHER RACES OR SPECIES? (MAP FROM WOOL'SRECENT SURVEY (Wool, Calvert, Constantino, Bellotti, & Gerling, In Press))

A survey of variation at an easily scored esterase locus in Columbia and adjacent localities uncovered sufficient geographic variation to suggest the presence of geographic races or distinct species (Wool, et al., In Press). Unfortunately no the loci were examined and it is difficult to sort out the status of these populations isolated by extremely high mountain ranges. Wool (1993), in a separate study has found that in Israel locality differences in the same esterase locus could be attributed to insecticide treatments rather than geographic isolation or host relationships.

SLIDE 22 FURTHER INFORMATION NEEDED

1. **What is the genetic basis for the Esterase phenotypes (mono- or polygenic, X-chromosome linkage)?**
2. **What is the incidence of parthenogenesis?**
3. **Where does the poinsettia or *B*-biotype (= species) come from? Need broadly based phylogenetic**

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Bemisia tabaci : Biotype or species complex?

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The term biotype when applied to insect parasites or parasitoids is generally used to distinguish between two or more morphologically similar or indistinguishable taxa that differ from one another in a biologically important way such as host preference, emergence time or some other ecological or behavioral trait. Unfortunately, several kinds of taxa and levels of genetic differentiation, outlined in Box 1, masquerade under the term biotype (see Diehl, 1984for examples, details and citations discussed in this article). Inevitably, the actual biological status of each biotype must be accurately established or efforts at control, quarantine or conservation will likely be difficult if not unsuccessful. To illustrate these differences, I present several examples and then review the status of the recent sweet potato white fly, biotype of *Bemisia tabaci*, now devistaing many crops in the New World.

Polyphenisms, sometimes called phenocopies or ecomorphs, *occur when identical genotypes produce distinct phenotypes under different environmental conditions.* There are several different types Diehl & Bush 1984. The solitary and gregarious "phases" of migratory locusts are examples of **seasonal polyphenisms**. Each phase, which differs in color, morphology and behavior, develops in response to crowding, change in food quality or other environmental cues. Seasonal polyphenisms induced by changes in temperature, photoperiodism and host plant quality are also

commonly encountered in many aphids. In temperate regions, early season forms are usually wingless (apterous) and reproduce parthenogenetically, while late season forms are frequently winged (alate) and reproduce sexually. **Host induced polyphenisms** may result in marked morphological differences in some scale insects and aphids when they feed on alternative hosts plant species. **Learning induced behavioral polyphenisms** have been observed in several insects, such as the apple maggot fly, *Rhagoletis pomonella*. Adult host preference and oviposition behavior depends on which host plant the fly first oviposits on or to which it is exposed during maturation (Papaj and Prokopy 1986). Although such changes in behavior are sometimes subtle, they may have a profound effect on the biology and host relationships of a population. These examples represent only a small sampling of the many kinds of polyphenisms that may occur in insects and other parasites.

Genetic polymorphisms, are *discontinuous phenotypes among individuals within a freely interbreeding population that are the result of allelic variation at a frequency higher than can be maintained by recurrent mutation*. For example, host plant specific polymorphisms may be caused by allelic variants of a single gene. The Hessian fly, *Mayetiola destructor*, is composed of populations each of which can only infest one or two cultivars of wheat. A gene-for-gene interaction appears to operates between each virulent Hessian fly "biotype" and its preferred susceptible wheat cultivar. A dominant resistant gene in the wheat cultivar is overcome by flies homozygous for a recessive virulent allele. Other examples are reported in aphids, scale insects and gall wasps. Insecticide resistance, similar to the kind of resistance Hessian flies display against

cultivar antibiosis, also often involve simple genetic polymorphisms. Many other examples involving morphological polymorphisms controlled by a single gene or "super gene" (several tightly linked genes that behave as a single trait), such as those involved in mimicry and industrial melanism, are well documented.

It is much more difficult to make clear distinctions between the remaining categories. This is because they represent somewhat arbitrary levels of increasing genetic differentiation and biological isolation between sister (genealogically the most closely related) populations. The levels of differentiation are imprecise and often difficult to establish because the same degree of genetic differentiation may have profoundly different effects on the degree of interaction between sister populations and their ability to interbreed. Only a few genetic changes in key traits may be sufficient to inhibit interbreeding between populations while other, often geographically isolated populations, may differ greatly in phenotype and genotype and still successfully interbreed when sympatric. Whether sister populations are classified as host races or different species depends more on qualitative rather than quantitative genetic differences and how these differences affect gene flow and the maintenance of biologically significant phenotypic distinctions. This means that the biological status of closely related populations exploiting different host relationships must be determined on a case-by-case basis. Accurate determinations require both a substantial knowledge of the biological as well as the genetic differences between the host associated populations. The outcome of a successful control or eradication program may rest on whether host races or distinct species are involved. The former may reestablish a new population on a

host from which a population has been eradicated while a species is less likely to do so.

A host or habitat race (ecotype, ecological race, biological race) *is a population of a species that is partially reproductively isolated from other conspecific sympatric sister populations as a direct consequence of adaptation to a specific host or habitat* (Diehl and Bush 1984). Different host race populations are sympatric if they occur within the cruising range of one another while in breeding condition (Mayr 1963). They may or may not evolve into species (see below) depending on the kind of stochastic environmental and biological conditions they experience during the course of their evolutionary history. The following criteria can be used to identify host races (Jaenike 1981; Bush 1993). (1) Individuals of different host-associated populations must be sympatric and in breeding condition; (2) Statistically significant genetic differences exist between sympatric populations over several generations; (3) Males and females exhibit genetic variation in host preference that results in host associated assortative mating; (4) Males and females show host-associated tradeoffs in fitness; (5) There is no evidence of post-mating reproductive incompatibility. Hybrid incompatibility indicates sibling species not host races.

Several examples of host and habitat or host races have been identified among Arthropods such as aphids, flies, moths and butterflies, mites, and parasitic copepods (Bush 1993; Bush submitted). Host races are restricted to groups in which mating behavior occurs only within or on a preferred host. In such groups genetic variation in habitat or host preference promotes the genetic differentiation between host associated populations,

and initiates host specific specialization and the maintenance of host races (Rice and Salt 1990; Bush 1993). The identification of host races usually requires extensive biological and genetic studies and is best accomplished on populations whose place and time of origin can be specified (Bush 1993). Because new host races appear infrequently and are difficult to recognize, they are often treated as host associated populations of a polyphagous species. Yet their biological characterization and genetic identification is essential for implementing sound pest management programs and for understanding the origin and evolution of new species.

The apple maggot fly, *Rhagoletis pomonella*. is an example illustrating how a host shift can result in the establishment of biologically distinct host races (Bush 1993). In 1862 *R. pomonella* was found infesting apples in a commercial orchard in the Hudson River Valley of New York. Soon afterward it was discovered infesting orchards in the surrounding states and within 50 years it had become a major pest of apples over the entire northeastern part of North America. These two sympatric (they share the same geographic area) races are morphologically indistinguishable in all life history stages. They are also interfertile and their hybrids are viable and fecund. However, they differ in genetically based traits such as host odor preferences, response to host odor, maturation rates, and eclosion times (their times of emergence in summer). Larvae and adults of both races also have consistently maintained highly significant differences in alleles frequencies in at least six loci from one generation to the next.

These genetic differences indicate that in nature the races are adapted to their respective host plants and do not freely interbreed. If appreciable hybridization did occur, then the races could not maintain these host

associated genetically based traits and would fuse into a single population. Because these differences between the races are maintained in all stages of development, it is clear that the adults are restricting their mating and oviposition to their preferred host. Mark-release-recapture studies over a three year period indicate the level of gene flow between the apple and haw races each generation has been estimated to be less than 6% (Feder, 1994).

The tradeoff in fitness comes primarily from selection acting on eclosion time which is under genetic control. If adults eclose prematurely and infest early ripening apples they may fail to diapause and will emerge late the same summer, too late to find fruit for oviposition or to complete development before frost. The haw race must time eclosion and maturation to coincide with a narrow window when haw fruits are in suitable condition for oviposition and before it is too late to complete development. Another factor that contributes to fitness differences between the races is the fact that apple flies escape the heavy parasitism experienced by the haw race. This provides a selective advantage in that it may compensate somewhat for the loss in fitness apple flies may experience feeding on the new host.

The apple and hawthorn populations of *R. pomonella* meet all host race criteria. Whether they already have passed the critical genetic threshold for speciation, i.e. the point where they will continue on separate evolutionary paths until all gene flow is terminated, cannot be determined. However, *R. pomonella* has been introduced into western North America, possibly within the last 20 years. In California, Oregon and Washington it infests haws and in some places apple. In Utah it primarily infests haw

and cherry, but not apple, while in Colorado it infests only haw, leaving the apple and cherry uninfested. The reason for the differences in its host preferences in these regions is unknown, but it is clear that these host races are somewhat labile and their future evolution is unpredictable.

Even more difficult to characterize and differentiate are geographic races and species. **Geographic races** (semispecies or subspecies) are generally regarded to be *geographically isolated populations not sufficiently different from one another to ensure reproductive isolation if they become sympatric.* Although phenotypically distinct in some way, such populations may or may not be associated with different habitats or hosts. It is assumed that if they were to establish contact they would interbreed and fuse into a single population. **Species**, on the other hand, *are natural sympatric or geographically isolated populations sufficiently reproductively isolated from one another to follow their own distinct independent evolutionary paths.* If sister species are geographically isolated it is assumed that they would not fuse upon contact even though some interbreeding and gene flow may occur from time to time.

Unfortunately these categories, although conceptually simple, are impossible to apply with certainty to most genetically and/or morphologically distinct closely related geographically isolated sister populations. There is no problem when populations are sympatric yet retain their distinct biological traits and between which little or no gene flow occurs. Such populations are distinct species. The only way to unequivocally establish if geographically isolated sister populations are

races or species is to experimentally test whether they would fuse upon contact or would maintain their distinct genetic and morphological traits even in the face of some hybridization. Such tests are almost impossible to carry out under natural conditions, particularly if one is dealing with a pest species. Single pair hybridization laboratory experiments designed to test for the ability to interbreed are notoriously unreliable predictors of taxonomic status. If crosses between individuals within a population are normal, but attempts to hybridize representatives from different populations fail, then clearly the two allopatric populations are distinct species. However, such a clear distinction cannot be made when all crosses are normal or nearly so. Such evidence is not sufficient to conclude that they would interbreed once they became sympatric as other factors are not considered which may actually contribute to or be responsible for keeping populations from fusing if and when they establish contact. For instance, sister populations can often maintain all their "species" characteristics even if gene flow occurs as a result of hybridization (Howard 1992). They may mate preferentially in one habitat or another and seldom actually contact one another during the critical mating period. Without the natural test of sympatry, or its reasonable simulation, there is no simple, direct way to ascertain if geographic races have passed the genetic threshold beyond which the populations would continue to diverge even in the face of gene flow. Just what constitutes such a threshold has yet to be established for any speciation event (Coyne 1992). Determining the biological status of geographically isolated populations is therefore often subjective and will probably remain so until a more objective criteria are developed.

Establishing the status of host-associated populations. With these conceptual and operational limitations of resolving various levels of biological differentiation in mind, how does one go about establishing the status of a newly recognized biotype? Obviously the most unambiguous and easily resolved case is when the old and new biotypes are sympatric and there is no evidence of interbreeding or gene flow. The two populations are distinct species. The presence or absence of gene flow is most easily and accurately accomplished by using biochemical (allozyme) or molecular (mitochondrial or nuclear DNA) markers. If each sympatric host associated sister population possesses unique alleles at one or more loci, and these differences are maintained over several generations, then the two populations are distinct species.

Distinguishing between host races and host-associated sibling species, however, is sometimes not so clear cut. How do you classify populations between which some gene flow can be demonstrated? We know, for instance, from research on *Drosophila* (Carson and Kaneshiro 1989) that gene flow may occur between sister species at a "low" level (1-3%) with no obvious effect on the maintenance of many species specific traits. In such cases the genes moving between populations appear to be neutral while selection removes those alleles that reduce fitness or affect essential species specific traits. If it can be demonstrated that most hybrids are the result of back crosses and not F_1's resulting from direct crosses between populations, then it is reasonable to assume the populations in question represent species not host races.

But what about higher levels of gene flow where F_1's occur at reasonable frequency? Gene flow between recently established hawthorn and apple

populations of the *Rhagoletis pomonella* has been estimated to be at most 6%. Are these populations species or host races? This is a difficult call and for now must remain somewhat subjective. The apple and haw populations maintain distinct host associated traits such as eclosion time, host preference, response to host odor, maturation rates, rates of parasitism, ability to learn to recognize alternate host fruit as well as allele frequencies at six allozyme loci. Because they are able to maintain these differences in the face of considerable gene flow, they behave as "good" species. Yet we have no way of predicting their evolutionary future; whether they will continue to accumulate genetic differences and experience progressively lower levels of gene flow or if, in response to environmental changes, they fuse into a single polyphagous species. Considering their recent origin and lack of recognized fixed genetic markers, it is probably prudent to regard them as well developed host races well on their way to becoming distinct species.

When populations are not sympatric then cross-matings should be attempted under conditions that approach as closely as possible those encountered by the populations in nature. This requires thorough knowledge of their biology and natural history to carry out such experiments properly. Sterility or reduced viability indicates the populations represent distinct species. When the crosses result in fertile offspring, F_2's and back crosses, however, it is seldom clear if the populations would interbreed in nature, particularly if they are associated with different hosts, and host associated cues responsible for mate recognition have not been provided. If crosses cannot be made the status of isolated populations must remain tentative. Until biological and genetic

criteria are established for pinpointing the threshold between race and species, only subjective inference on the status of populations in these early stages of speciation can be made.

Establishing the biological status of closely related sister populations, such as host races, is not only of academic interest, but is of practical necessity as well. Effective, long term control strategies may rest on whether only one or more species are involved. For instance, is it necessary to treat a pest population infesting only one host or must all populations of the same species associated with different hosts be controlled? Does each host race represent a potential source of individuals genetically preadapted to recolonize a pest free economically important plant or can they be ignored? Unfortunately, this problem is not easily resolved without extensive biological research.

The *Bemisia tabaci* Biotype Problem: What is the status of the cotton (A) and poinsettia (B) biotypes reported for *B.tabaci*? The former has been a pest of several vegetable crops in the western hemisphere at least since 1894 while the latter was first noted in 1986 (Perring, Cooper et al. 1993). First, I will discuss the results of recent research on the biology of this species. This evidence can then be used to evaluate the biological status of host associated *Bemisia* populations based on the criteria outlined above.

Biological differences: (1) The B biotype rapidly replaced A biotype in southwestern USA after its introduction to that region (Perring, Cooper et al. 1993). (2) Biotype B has much wider host range than biotype A (Brown 1993) . (3) Single pair matings between strain A and B biotypes produce no female offspring whereas crosses between individuals from the same

strain produce both males and females (Perring, Cooper et al. 1993). Because white fly males develop from unfertilized eggs and are haploid, females are diploid as they are produced only from eggs that have been fertilized eggs (haplo-diploid). Inter-strain crosses therefore can produce males even if no copulation occurs. Even if copulation occurs eggs may fail to develop either because they were not fertilized or the embryo did not survive because of genome incompatibility. (4) The A and B biotypes transmit different viruses. For instance, biotype B transmits silver leaf virus while biotype A does not (Perring, Cooper et al. 1991).

Genetic differences: The A and B biotypes differ in several distinct genetic traits. (1) Each biotype has one or more unique alleles at six loci not found in the other and they show fixed allelic difference at 4 loci (Perring, Cooper et al. 1991; Perring, Cooper et al. 1993) . (2) Each biotype expressed a unique set of seven amplified PCR (polymerase chain reaction) products using RAPD primers (Perring, Cooper et al. 1993). Similar results are reported by Gawel (1993) . (3) No heterozygotes for known genetic markers unique to each biotype have been clearly demonstrated in natural sympatric populations of the A and B biotypes. In fact, as emphasized above, it appears that biotype B has replaced A in most areas of southwestern USA (Perring, Cooper et al. 1993).

On the basis of esterase banding patterns, Brown (1993) concluded that the B biotype had spread throughout Central and probably South America. Her data also suggest that other, previously unrecognized biotypes may also exist in these regions. Another study has noted further population subdivision of *B. tabaci* within a region. Wool (In Press), also using a single esterase marker, noted differences between geographically isolated

populations within Columbia. Unfortunately, variation noted in a single enzyme locus is not sufficient for drawing conclusions about biotype status or geographic subdivision. This is particularly true of esterases which are often subject to rapid changes in response to local environmental conditions and insecticide applications.

Conclusions on the Status of *Bemisia tabaci* Biotypes: Clearly the A and B biotypes, at least in the USA, meet all the criteria of distinct, non-interbreeding species. Each A and B biotype has its own host range which has probably evolved independently over time throughout the middle latitudes in geographically isolated populations. Each one of these biotypes may in turn represent either a complex of host races or cryptic sibling species. Only detailed genetic and biological studies of representative populations from throughout the world can resolve their actual biological status. With the advent of rapid transportation and an increase in commercial traffic in ornamentals and other plants, geographically isolated populations *B. tabaci* appear to be widely and indiscriminately introduced and interchanged throughout the tropical and subtropical regions.

A survey shoud be undertaken to establish the biological status and phylogenetic relationships among and between the *B. tabaci* populations now spread worldwide. Such a survey, preferably based on molecular traits such as mitochondrial and nuclear DNA sequences, could help resolve the origin and pattern of movements of taxa in the *B. tabaci* complex . Becuase it is possible to amplify DNA sequences from insects preserved for years in alcohol or dried on a pin, museum specimens collected long ago could provide information about past population

structure and distribution. Although RAPD-PCR is not suitable for phylogenetic studies because homology between amplified products is difficult to establish, this technique is useful for distinguishing between pairs of closely related sister species and could help establish if two populations with distinct biological characteristics represent distinct species. Allozyme electrophoresis can also be used to establish relationships, but it does not have the resolving and analytical power of the molecular techniques now in use.

The resolution of other problems would help clarify the origin and pattern of evolution of the *Bemisia* complex. The genetic basis for the esterase phenotypes (mono- or polygenic, X-chromosome linkage), as well as the other allozyme markers used to identify the A and B species, should be established if they are to be used for further population genetic studies. A clear understanding of the incidence and genetic basis for parthenogenesis is required if more detailed research into the genetics of host preference and virus transmission are to be undertaken. Finally, the genetic basis for interspecific incompatibility (mate recognition, genomic or cytoplasmic incompatibility or combinations thereof) should be determined. Knowing the cause of reproductive isolation between species A and B would help to establish whether new host races and species can develop sympatrically and how rapidly they can evolve allopatrically.

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Table 1

BIOTYPE LOOK ALIKES

1. Non-genetic polyphenisms

2. Polymorphic or polygenic variation within populations

3. Habitat (host) races

4. Geographic races

5. Species