Successive Microbial Populations in *Calimyrna* Figs

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ABSTRACT

Miller, M. W. (University of California, Davis) and H. J. Phaff. Successive microbial populations in *Calimyrna* figs. Appl. Microbiol. **10**:394–400. 1962.—Smyrna-type (*Calimyrna*) figs have essentially sterile internal tissue until visited by the pollinating fig wasp, *Blastophaga psenes*, which introduces a specific microflora consisting of *Candida guilliermondii* var. *carpophila* and *Serratia plymuthica*. This flora persists and develops in numbers throughout the ripening period until maturity of the fruit. These organisms do not cause spoilage. The presence of *C. guilliermondii* var. *carpophila* appears to increase the attractiveness of the fruit to drosophilae. *Drosophila* (mainly *D. melanogaster*) carry spoilage yeasts and bacteria on their exterior body parts, and introduce these organisms during ovipositing in the fruit cavity. The spoilage yeasts consist almost entirely of apiculate yeasts (*Hanseniaspora valbyensis*, *H. uvarum*, and *Kloeckera apiculata*) and of *Torulopsis stellata*, which cause active fermentative spoilage. Spoilage bacteria (primarily *Acetobacter melanogenus*) are also introduced with the yeasts. Organic acids are produced by these yeasts as well as by the *Acetobacter*. A number of minor spoilage yeasts were also identified.

One of the problems of the fig industry is microbial[1] spoilage while the fruit is still on the tree. Fermentative spoilage, or "souring" as it is termed by the industry, is one of the more common types and is caused by both yeasts and bacteria (Mrak et al., 1942).

Figs are unique in that the "fruit" is a hollow structure lined with flowers,. i.e, a syconium. The important commercial Smyrna-type of fig (called *Calimyrna* variety in California) differs from other commercial varieties in that it contains only pistillate flowers and that it must be pollinated. Pollination is necessary for the seeds to form, to insure proper setting of the fruit, and to prevent it dropping from the tree prematurely. Pollination is effected by the fig wasp (*Blastophaga psenes* Linneaus), which carries pollen from the inedible caprifig to the *Calimyrna* fig. To accomplish this, perforated bags containing caprifigs are placed in each *Calimyrna* tree. Full details of the life cycle of the fig wasp and its role in pollinating *Calimyrna* figs have been described by Condit (1947).

The normal microflora found in the caprifig, and that associated with the fig wasp, was studied by Phaff and Miller (1961). They found that this microflora consisted of a single bacterial species, *Serratia plymuthica* (Lehmann and Neumann) Bergey et al., and a single species of yeast, *Candida guilliermondii* (Castellani) Langeron and Guerra var. *carpophila* Phaff and Miller.

It was shown that the fig wasp introduces these microorganisms into the *Calimyrna* variety during pollination. Despite a limited multiplication in the interior of the fig, these organisms do not cause a detectable fermentative spoilage or souring. When caprifigs are improperly handled by the grower, they may become infected with mold. As a result the fig wasp may also carry spoilage molds to the edible figs. One of the most troublesome molds carried by the fig wasp is *Fusarium moniliforme* Sheldon var. *fici* Caldis (Caldis, 1927). This fungus causes an internal rot of the syconium known as endosepsis.

Mrak et al. (1942) isolated 115 cultures of yeast from soured figs which represented species of *Saccharomyces*, *Pichia*, the apiculate yeasts (*Hanseniaspora* and *Kloeckera*), *Candida*, and *Torulopsis*. Since no quantitative estimations were made of the relative proportions of the various species isolated by these investigators, no conclusions can be drawn as to the most significant species responsible for spoilage of the figs. Miller and Mrak (1953) collected dried-fruit beetles, *Carpophilus hemipterus* Linn., from the interior of souring figs and isolated, by direct plating, the yeasts adhering to the exterior body parts and those found in the intestinal tracts. Approximately the same yeast flora was found on the exterior of the beetles as in their intestinal tract. This indicated that the beetles were feeding on the fermenting-fig tissue. The most common organisms were apiculate yeasts (*H. valbyensis* and *K. apiculata*), *Candida krusei*, and *C. guilliermondii* var. *carpophila* (called *T. carpophila* by Miller and Mrak, 1953). It is significant that only a single isolate of one species of *Saccharomyces* (*S. rosei*) was found. Unfortunately, relative proportions of the populations of the various species were not estimated.

The present work is a continuation of our previous study, which demonstrated that the fig wasp introduces a specific yeast and a specific bacterium into *Calimyrna* figs, and that these microorganisms do not cause spoilage. Evidence will be presented showing that figs which are visited, subsequent to pollination, by *Drosophila* (for the primary purpose of oviposition) become inoculated with other yeasts and bacteria which rapidly cause fermentative spoilage or souring.

Materials and Methods

Pollinated *Calimyrna* figs and figs of the Adriatic variety were obtained from three well-separated orchards in the Fresno area of California.

All samples of figs were carefully inspected with a wide-field microscope for the presence of eggs or larvae of the vinegar fly, *D. melanogaster* Meigen, and of the dried-fruit beetle *C. hemipterus*. In opening a fig the exterior surface was cleaned with 70% ethanol, flamed momentarily, and a longitudinal slit was made through the skin only (starting in the area of the eye) with a sterile scalpel. The cavity was exposed by tearing the flesh along the incision. Portions of the fig tissue were removed near the eye and from several other locations within the cavity. The material from one-half of the fig was mixed thoroughly; one large loopful was streaked evenly over the surface of a 10% malt agar plate (pH 6.4). Tissue from the other half was treated similarly, but was plated on 10% malt agar (pH 3.5) to reduce bacterial growth. The pH was adjusted with 1 N HCl immediately before pouring the plates.

When the yeast flora of a *Drosophila* crop was analyzed, the procedure described by Phaff et al. (1956*a*) was followed. Identification of the bacteria was done with the aid of *Bergey's Manual of Determinative Bacteriology* (Breed, Murray, and Smith, 1957). The procedures of Lodder and Kreger-van Rij (1952) were used to identify the yeasts, except that the number of carbon compounds in assimilation tests was increased to 34 (Wickerham, 1951). Carbon-assimilation studies were made by the use of a multipoint inoculating device (Beech and Carr, 1955) with carbon compounds incorporated in Yeast Nitrogen Base (Difco), using flat-bottomed Petri dishes.

Mashed bananas were used for determining differential attraction of the various microflorae to *Drosophila*, since *Calimyrna* figs with sterile tissue were difficult to obtain. The banana pulp was placed in 4-oz bottles, sterilized for 10 min at 110 C, and inoculated. All combinations of cultures which did not include *Acetobacter* were grown for 48 hr. In those including *Acetobacter*, the other organisms were grown 24 hr before inoculating the *Acetobacter*. The bottles were then incubated for an additional 24 hr before exposure to the flies. A total of 18 samplings was made of each combination during the four trials.

If very immature *Calimyrna* figs are sprayed with sodium salt or the butyl ester of benzothiazolyl-2-oxy acetic acid, the fruit will remain on the tree and mature in the absence of pollination. In such figs, seeds are not formed. These hormone-treated figs would not be expected to contain the microflora normally introduced by the fig wasp during pollination. Several samples of such fruit were obtained from one experimental orchard.

Some hormone-set figs were inoculated by introducing suspensions of the various cultures through the eye of the fig with a syringe. The fruit samples were placed in 16-oz wide-mouthed bottles and incubated for 48 hr. Six samplings were made during these tests.

The bottles were placed in an odor-free room (8 by 12 feet) with a low light intensity, and a large number of laboratory-reared *D. melanogaster* were released in the room. Flies trapped at each sampling were removed from the test room and counted.

The data were statistically analyzed by analysis of variance with unequal proportionate numbers (Snedecor, 1956) or by least significant differences (Alder and Roessler, 1958).

Results

Microflora of sound figs. First, a survey was made of mature insect-free sound *Calimyrna* figs on the tree to confirm the presence of the previously established microflora introduced by the fig wasp during pollination. In 102 of 147 fruits (69%), the flora consisted essentially of *S. plymuthica* and *C. guilliermondii* var. *carpophila*, whereas the remaining 31% were sterile. Occasionally, *F. moniliforme* was also present. These results agree with the data previously published (Phaff and Miller, 1961). Apparently, when the fruit is lightly pollinated, the characteristic microflora does not always develop. Comparable samples of the Adriatic fig variety, which does not require pollination, showed that 82% of the figs had sterile internal tissue. The microflora of the remaining 18% was different from that of the *Calimyrna* variety and may have been introduced by insects other than stray fig wasps.

Change in microflora after visitation by insects. During the next season, a series of samples of *Calimyrna* figs was taken during the harvest season in three well-separated fig orchards in the Fresno area. Part of the samples were those whose internal cavity, upon inspection with a wide-field microscope, was sound and completely free of any evidence indicating visitation by insects other than the fig wasp. Comparable figs were sampled where there was evidence of insect infestation (Table 1). The most common insect in the fig trees was the vinegar fly, *D. melanogaster*. To a lesser extent the dried-fruit beetle, *C. hemipterus*, was also present. The microflora of the normal figs was typical of that brought in by the fig wasp. On the other hand, figs which had been visited by *Drosophila* or by dried-fruit beetles presented a striking change in microflora. Evidence of insect visitation was based on oviposition in the interior portion of the fig, near or at the opening or "eye," or on the development of larvae in the fleshy moist tissue. Occasionally, figs were collected showing no evidence of eggs or larvae, although adults were seen to emerge through the eye upon picking the fruit. The extent of the change in microflora from a normal one to a flora including typical spoilage organisms was more or less proportional to the intensity of oviposition and to the degree of larval development.

This correlation is shown in more detail in Table 2,

which contains a description of the condition of a number of individual figs and the kinds and approximate proportions of microorganisms developing on the plates streaked with fruit tissue. Of the first five figs, which apparently were visited only by dried-fruit beetles, three did not show a change in their bacterial and yeast flora due to visitation by the beetles, as it remained the same as that found in the tissue of normal pollinated figs. The other two figs of this group showed the normal *Serratia* flora, but *C. guilliermondii* var. *carpophila* was largely overtaken by growth due to apiculate yeasts. The occurrence of black mold in three of the figs may be explained by the fact that the dried-fruit beetle is a soil inhabitant and, thus, is likely to carry spores of the common spoilage molds from soil to the fruit.

The remaining samples were infested by *Drosophila*. With these, a trend was observed, indicating that the greater the severity of infestation the more the normal microflora was reduced or eliminated in favor of the spoilage flora. The latter consisted of two principal kinds of yeast (*T. stellata* and the apiculate yeasts) and several minor species. The complete scientific names of all the yeasts isolated and identified are included in Table 3. In figs showing only egg deposition by *Drosophila*, *C. guilliermondii* var. *carpophila* and *S. plymuthica* were still present. As the spoilage progressed, this flora was completely replaced by the spoilage yeasts and by vinegar bacteria, of which several isolates were identified as *A. melanogenus* Beijerinck 1911. The vinegar bacteria convert alcohol produced by the yeasts to acetic acid. In addition, the apiculate yeasts and *T. stellata* form noticeable amounts of acid from glucose on chalk agar plates, confirming earlier studies by Mrak et al. (1942) with fig juice as the substrate. The combined acidic metabolic products are responsible for the term "souring" and may well be the main cause for the complete elimination of *Serratia* from figs during advanced stages of spoilage.

Role of Drosophila in introducing spoilage flora. Thus far, our evidence, that *Drosophila* were mainly responsible for the introduction of a spoilage flora in figs, was based primarily on the correlation between the simultaneous

TABLE 1. *Change in microflora of mature Calimyrna figs after visitation of the fruit cavity by insects**

Figs	Orchard designation	Number of samples	*S. plymuthica* only	*C. guilliermondii* var. *carpophila* only	*Serratia* and *Candida*	No growth	*Fusarium* present also	Spoilage yeasts and bacteria present
Normal, pollinated	A	35	16†	0	15	4	2	0
	B	18	5	0	10	3	1	0
	C	33	7	3	12	11	1	0
Figs showing evidence of insect infestation (eggs, larvae, or adults)	A	9			V‡	0	2	9
	B	10			V	0	0	10
	C	3			V	0	0	3

* Samples were collected during the harvest period from three different orchards (A, B, and C).

† The figures indicate the number of figs yielding the flora listed.

‡ Variable percentages of *S. plymuthica* and *C. guilliermondii* var. *carpophila* present in the samples depending on the extent of infestation and progress of spoilage.

TABLE 2. *Microbial flora present in Calimyrna figs from which dried-fruit beetles or Drosophila were seen to emerge**

Sample number and description of fig samples†	Microorganisms present						
	C. guilliermondii var. *carpophila*	*Serratia plymuthica*	Apiculate yeasts	*Torulopsis stellata*	Spoilage bacteria (mainly *Acetobacter*)	Misc. yeasts (*Candida*, *Pichia*, etc.)	*Fusarium*
131	N	N					N
132	N	N					N
133	N	F					‡
111	F	N	N				‡
112		N	N				‡
143 No eggs or larvae detected			N		M	M	
267 Few eggs around outside of eye; no larvae present	N	N	F				
134 Few eggs in eye area; no larvae present	M		M				
116 Moderate number of eggs in fig; no larvae present	M	M	N			F	
138 Similar to 116	M		N		F	F	
142 Similar to 116	F		N		F		
118 Numerous eggs but no larvae present in fig	F	N	N				
119 Similar to 118		M	M			N	
137 Similar to 118			N	M	F	F	
140 Similar to 118			N	M	N	F	
115 Numerous eggs and larvae present in fig		F	N			F	
135 Similar to 115	F		N	M	N		
139 Similar to 115			M	N	M	F	
141 Similar to 115	M		M	M	F		

* F = few (less than 15 colonies per plate); M = moderate (15 to 50 colonies per plate); N = numerous (more than 50 colonies per plate).

† First five samples are figs from which dried-fruit beetles were seen to emerge, with no evidence of adult *Drosophila*, eggs, or larvae; fig tissue not obviously spoiled. Adult *Drosophila* were seen to emerge from all other fig samples recorded here.

‡ Samples containing *Aspergillus niger* or molds other than *Fusarium*.

detection of spoilage organisms and evidence of *Drosophila* infestation. The next experiments were designed to obtain additional proof for this assumption.

Drosophila are known to be attracted to certain fermented substrates (*see* Dobzhansky et al., 1956). It is also known from several studies that the flies feed upon these substrates, since fermentative yeasts are found regularly in their crops (Shehata et al., 1955; Phaff, Miller, and Shifrine, 1956*b*). Other substrates, such as fermenting tomatoes, appear to be used both for feeding and for oviposition (de Carmargo and Phaff, 1957). During feeding or ovipositing, it may be expected that the external body parts become contaminated with microorganisms. The yeasts which are consumed by the flies are ultimately digested and serve as food components (Shehata and Mrak, 1951). Thus, a fly migrating into a fig orchard is likely to carry spoilage organisms on its exterior body parts. If feeding occurred recently, the crop contents might also be expected to contain the spoilage yeasts.

To obtain information on the development of the spoilage flora in figs as related to visitation by *Drosophila*, sterile test tubes were held against the eyes of large numbers of figs on the tree and the fruit was tapped gently by hand. If flies emerged from the fruit they were collected in the test tube and the fruit itself was removed from the tree. Next, the fruit was opened aseptically, inspected with a wide-field microscope, and the tissue was plated. Then the crop was dissected from one of the flies and similarly plated. The yeasts which grew were estimated with respect to population distribution and representative colonies were purified and identified (Table 4).

TABLE 3. *Taxonomic designation and relative frequencies of the yeasts isolated from soured Calimyrna figs on the tree and from crops of Drosophila melanogaster emerging from such figs**

Taxonomic designation	Yeast isolates from figs infested by drosophilae				Yeast isolates from *Drosophila* crops			
	Total	F	M	N	Total	F	M	N
Saccharomyces veronae Lodder and Kreger-van Rij, 1952	1	—	1	—				
Pichia kluyveri Bedford, 1942	5	2	3	—				
Pichia sp.	3	2	1	—				
Hanseniaspora valbyensis Kloecker, 1912	6	—	3	3				
H. uvarum (Niehaus) Shehata et al., 1932	14	3	1	10	5	1	2	2
Kloeckera apiculata (Reess *emend.* Kloecker) Janke, 1870	8	—	1	7	2	—	2	—
Combined apiculate yeasts	(28)	(3)	(5)	(20)	(7)	(1)	(4)	(2)
Torulopsis stellata (Kroemer and Krumbholz) Lodder, 1931	13	—	7	6	13	1	2	10
Candida krusei (Cast.) Berkhout, 1910	5	4	1	—	2	2	—	—
C. guilliermondii (Cast.) Lang. and Guerra, 1912	1	—	1	—	—	—	—	—
C. guilliermondii (Cast.) Lang. and Guerra var. *carpophila* Phaff and Miller, 1961	15	2	9	4	1	1	—	—
Total	71				23			

* F = few (less than 15 colonies per plate); M = moderate (15 to 50 colonies per plate; N = numerous (greater than 50 colonies per plate).

In fig no. 205, which contained no eggs or larvae, the microflora of the flesh was normal, consisting of *S. plymuthica* only. However, the crop contents of the fly trapped from the fig contained the typical spoilage flora, i.e., apiculate yeasts and *T. stellata*. Presumably, the two flies present in this fig had entered shortly before the collection was done, and they had not as yet oviposited. The spoilage yeasts would not have had time to reach detectable numbers. These particular data, however, support the previously cited evidence that the *Drosophila* introduce the spoilage flora. As the extent of infestation (eggs and larvae) and spoilage signs increased, the flora of the fig tissue and that of the crops of visiting drosophilae became more alike, although certain differences were noted. *T. stellata* was found consistently in the *Drosophila* crop; it was generally present much more abundantly than the apiculate yeasts, which were even absent occasionally. In the fruit tissue the opposite trend was observed; the apiculate yeasts were generally most numerous. These findings and the very rare occurrence of *C. guilliermondii* var. *carpophila* (representing the normal yeast flora of sound figs) in the *Drosophila* crop indicate that the flies primarily visit the fig syconium for the purpose of ovipositing rather than for feeding.

Attractivity studies. An attempt was made to determine whether or not the metabolic activities of the microflora introduced by the fig wasp might be responsible for making *Calimyrna* figs more attractive to *Drosophila* than figs containing essentially sterile internal tissue.

Preliminary studies using mashed banana and mashed banana inoculated in various combinations with *S. plymuthica*, *C. guilliermondii* var. *carpophila*, and *A. melanogenus* (as a souring bacterium) established that the basic substrate alone (banana) was not attractive to drosophilae (0.05 probability level). Banana inoculated with the carpophila yeast, alone or in combination with *Serratia*, was the most attractive of the various samples (0.001 probability level). There was no significant difference in the attractivity of the other five combinations. It is of interest to note that *A. melanogenus* decreased the attraction of the combinations containing yeast to the same level as that of the bananas inoculated with these bacteria only. Statistical data are summarized in Table 5.

Tests were made in which hormone-set *Calimyrna* figs were used as a basic substrate, to avoid complications with the *Serratia-Candida* flora of pollinated *Calimyrna* figs. The spoilage yeasts *H. valbyensis*, *H. uvarum*, and *T. stellata* were tested, in addition to *C. guilliermondii*

var. *carpophila*, to determine relative attractivity. The limited data obtained in these tests showed that figs inoculated with either *H. valbyensis* or with *T. stellata* were more attractive than uninoculated figs (0.05 probability level). Figs inoculated with *H. uvarum* or with *C. guilliermondii* var. *carpophila* were more attractive than uninoculated figs, but the probability level did not reach 0.05 (Table 6).

These limited tests suggest further studies to obtain additional information on the mechanism(s) by which *Drosophila* are attracted to figs.

Competition between C. guilliermondii var. carpophila and yeasts causing spoilage. It is curious that the carpophila yeast, although it ferments glucose and fructose, did not cause a noticeable alcoholic fermentation of the moist fig tissue, whereas the apiculate yeasts and *T. stellata* caused rapid fermentation and spoilage once they were introduced in the fig tissue. Analyses of *Calimyrna* figs by Mohamed and Mrak (1942) indicated approximately 72.7% moisture and 18% sugar in fruit ready for harvest. To determine whether the sugar level of the fruit might be a factor in favoring the selection of spoilage yeasts over the carpophila yeast, a number of liquid media were prepared with sugar contents of 16°, 27°, and 38° Brix. The basal medium was 10% malt extract to which glucose was added to reach the desired Brix level; 5 ml of medium were placed in test tubes containing inverted vials. The tubes received a light inoculum from malt agar slants. Five strains each of *H. valbyensis*, *H. uvarum*, *T. stellata*, and *C. guilliermondii* var. *carpophila* were used. In the 16° Brix medium, full vials of gas were obtained in 2 days with all yeasts except with the carpophila yeast, which required 4 days. When full vials were observed, one drop of each culture was transferred to the 27° Brix media. Full vials were produced in 3 days by the spoilage yeasts, whereas the carpophila yeast required 6 days. In 38° Brix media, *T. stellata* and *H. valbyensis* produced full vials of gas in 5 to 8 days, whereas *H. uvarum* and the carpophila yeast fermented the sugar very slowly. Thus, spoilage yeasts may be expected to outgrow the carpophila yeast in maturing figs (see also Tables 2 and 4). It does not explain why the carpophila yeast alone does not cause spoilage in *Calimyrna* figs, as this yeast is perfectly capable of fermenting sugar solutions of 16 to 27° Brix and it is

TABLE 4. *Comparison of the microflorae from Calimyrna figs and from drosophilae (crop contents) trapped from the same fig**

Sample number	Description of fig samples	Microorganisms present				
		C. guilliermondii var. *carpophila*	*Serratia plymuthica*	Apiculate yeasts	*Torulopsis stellata*	Misc. yeasts (*Pichia*, *Candida*, etc.)
205	Fig tissue appeared sound; no eggs or larvae observed		N			
206	Fly			*M*	*M*	
203	Fig tissue appeared sound; few eggs and larvae present near eye area	M	N	N		
204	Fly				*N*	*F*
219	Fig green and soft; numerous eggs in eye area; few larvae present			N	F	
220	Fly			*N*	*F*	*F*
217	Fig yellowish green and firm; numerous eggs in eye area; few larvae present			N	M	
218	Fly			*F*	*N*	
213	Fig green, firm, but soured; very numerous eggs; moderate number of larvae in eye area	M		N		
214	Fly	*F*		*F*	*N*	
225	Fig tissue appeared sound; very numerous eggs and larvae present	M	N	N		
226	Fly			*M*	*N*	
215	Fig soured and dripping; very numerous eggs and larvae present			N	M	F
216	Fly			*F*	*N*	
229	Similar to 215				N	F
230	Fly				*N*	*F*
227	Fig soured; very numerous eggs; no larvae, but a few pupae present				N	
228	Fly				*N*	
207	Fig soured and dripping; very numerous larvae, no eggs present	F		M	N	
208	Fly				*N*	
209	Similar to 207			M	M	
210	Fly				*N*	
221	Similar to 207; (*Drosophila* larval development made colony estimation impossible)			—	—	
222	Fly			*F*	*N*	*F*
223	Similar to 207			N		
224	Fly				*N*	

* F = few (less than 15 colonies per plate); M = moderate (15 to 50 colonies per plate); N = numerous (greater than 50 colonies per plate). For better comparison, isolations from *Drosophila* are italicized.

introduced very early, i.e., during the pollination period. It is possible that the developing *Drosophila* larvae are mainly responsible for spreading the spoilage organisms and for damaging the internal fig tissue. In this way the sugar-containing cell sap may become exposed to the action of the fermentative yeasts and bacteria.

Taxonomy and frequency of occurrence of yeasts. During the entire study, a total of 94 yeasts was isolated from spoiled *Calimyrna* fig tissue and from the crops of *D. melanogaster*. Their taxonomic designations and their relative frequencies in the various samples are shown in Table 3. It is evident that the apiculate yeasts (*H. valbyensis*, *H. uvarum*, and *K. apiculata*) and *T. stellata* were the most important spoilage yeasts, both by numbers of isolates and population. In only one case, *C. krusei* constituted 45% of the colonies appearing on a plate streaked with tissue from a fig which contained numerous eggs. Other isolates occurred only in small or moderate numbers. *C. guilliermondii* var. *carpophila* was mainly numerous in fig tissue where insect infestation was very light. *K. apiculata* is the imperfect form of both *H. valbyensis* and of *H. uvarum*. *H. valbyensis* contains four hemispherical to hat-shaped ascospores per ascus and *H. uvarum* ordinarily has but one spherical spore per ascus. The assimilation and fermentation patterns of the three species are identical. Also their colony morphology is sufficiently similar so that the relative distribution between the three species cannot be stated with absolute certainty. Because of the difficulty of distinguishing these colonies on plates, Table 3 also contains the combined figures for the apiculate yeasts.

TABLE 5. *Attraction of various combinations of Serratia plymuthica, Candida guilliermondii var. carpophila, and Acetobacter melanogenus to Drosophila flies*

Substrate	$\bar{X}$*	Analysis of variance		
		Source of variation	Degrees of freedom	Mean squares
1. Banana	0.78			
2. Banana + *S. plymuthica*	7.55	Total	143	
3. Banana + *C. guilliermondii* var. *carpophila*	36.83	Samples	7	2,508.42†
4. Banana + *A. melanogenus*	6.56	Trials	3	734.08†
5. Banana + *S. plymuthica* + *C. guilliermondii* var. *carpophila*	22.44	Samples × trials	21	388.59†
6. Banana + *S. plymuthica* + *A. melanogenus*	7.11	Error	112	70.74
7. Banana + *C. guilliermondii* var. *carpophila* + *A. melanogenus*	6.94			
8. Banana + *S. plymuthica* + *C. guilliermondii* var. *carpophila* + *A. melanogenus*	7.00			

* Least significant difference: 0.05 = 5.56; 0.01 = 7.37; 0.001 = 9.48. $\bar{X}$ = mean number of flies attracted to substrate during the tests.

† Probability level = 0.001.

Discussion

Two striking changes may occur in the microflora of *Calimyrna* figs due to involvement of insects. The first change is a normal one and it occurs when the green sterile fruit is pollinated by *Blastophaga* wasps (Phaff and Miller, 1961).

The second change in microflora follows the visitation of the fruit by other insects during the later stages of ripening. These are mainly *D. melanogaster* and, to a lesser extent, *C. hemipterus*. Invariably, after the fruit was visited by *Drosophila*, fermentative spoilage and souring ensued. *Drosophila* appear to drift into the orchards from neighboring areas, such as peach orchards and similar habitats, when the harvest of these particular crops is completed. In their search for new breeding places, they enter the figs and continue their cycle of development. During ovipositing in the fig, the fruit is contaminated with yeasts and bacteria adhering to exterior body parts of the flies. During subsequent larval development, the larvae spread the contaminants throughout the flesh of the fig and active fermentative spoilage results.

The dried-fruit beetle appears to be a less important initial vector for spoilage organisms than is *Drosophila*. The beetle normally lives in the soil or in decomposing plant material. Ordinarily, it does not seem to carry sufficient spoilage yeasts and bacteria to initiate souring, unless it originated in a fig already undergoing fermentation (Miller and Mrak, 1953). The latter situation may occur during the final stages of ripening.

In the present work, particular attention was paid to the estimation of the population distribution of the significant spoilage organisms. This was possible owing to the peculiar characteristics of the colonies, which could

TABLE 6. *Attraction of the spoilage yeasts (Hanseniaspora valbyensis, H. uvarum, and Torulopsis stellata) and of Candida guilliermondii var. carpophila to Drosophila using hormone-set figs of the Calimyrna variety*

Substrate	$\bar{X}$*	Analysis of variance		
		Source of variation	D.F.	M.S.
1. Fig	1.83			
2. Fig + *H. valbyensis*	56.33	Total	29	
3. Fig + *H. uvarum*	24.83	Samples	4	2,395.45†
4. Fig + *T. stellata*	39.17	Error	25	680.77
5. Fig + *C. guilliermondii* var. *carpophila*	29.67			

* Least significant difference: 0.05 = 31.03. $\bar{X}$ = mean number of flies attracted to substrate during the tests.

† Probability level = 0.05.

be recognized with some practice. In many ecological studies reported in the literature, the isolates are merely listed by the number of strains of each species without indication of the proportions of the population of each species. Moreover, enrichment techniques simply result in the selection of the most vigorously growing organisms and lead to a distorted picture of the microbial flora originally present.

It should be kept in mind that direct plating of samples does not permit recognition of minority populations which comprise less than about 0.5% of the total population. On normal malt agar plates, the slimy growth due to *S. plymuthica* often covered the yeast colonies, but on acidified malt agar (pH 3.5) very few bacteria grew (mainly *Acetobacter*) and the yeasts were easily enumerated.

We have shown that the apiculate yeasts and *T. stellata* usually comprise at least 90% of the spoilage flora of figs. This finding is in contrast to those of Mrak et al. (1942) and Miller and Mrak (1953), where *Saccharomyces cerevisiae* and *C. krusei* were listed as the most numerous species present in spoiled figs. It is interesting that typical representatives of the genus *Saccharomyces*, such as *S. cerevisiae*, were virtually absent and therefore do not play a role in fruit spoilage. This indicates that the flies did not originate in winery pomace piles, one of their possible breeding places during the very late stages of the fig season. Although tomatoes mature after the fig harvest and therefore cannot be implicated as a source of *Drosophila*, it is worthy of note that the yeast flora of spoiled fermenting tomatoes did not include species of *Saccharomyces* either, but consisted of about equal proportions of *H. uvarum* and *Pichia kluyveri* (de Camargo and Phaff, 1957). The absence of *Saccharomyces* on fresh apples, cherries, strawberries, and grapes was noted by Sasaki and Yoshida (1959) in Japan, and by Clark, Wallace, and David (1954), and by Williams, Wallace, and Clark (1956) for cultivated and wild apples in Canada. Mooser (1958) reported the absence of *S. cerevisiae* on 30 samples of skins of European wine grapes.

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