mally, and this has proved to be the case.

It is concluded that the symptoms shown by the watermelon plants were brought about by varying amounts of 2,4-D absorbed by the roots. The absence of stem and petiole curvatures, the norm when surface application of 2,4-D is made to a plant, confirms this diagnosis. Further, the extreme care taken in application obviated contact of the weed killer with the foliage or stems of the melon seedlings.

We are presenting this paper to show that small quantities of 2,4-D can be absorbed by watermelon plants from the soil through the root-systems, when weed-killers are applied with the plants *in situ*. The same or a similar response may occur when the soil is sprayed to eradicate weeds, and melon seeds planted later. However, the injuries described are of a transitory nature only, for the affected plants grew out of the condition and flowered and fruited normally.

PROCESSING SECTION

COMPARISON OF PLATING MEDIA USED FOR THE ESTIMATION OF MICROORGANISMS IN CITRUS JUICES[3]

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The production of frozen concentrated citrus juices requires a certain amount of routine bacteriological control work, most of which is concerned with the common plate count. The significance of a "total" count on any particular sample of concentrate is extremely doubtful since, in many instances, it does not reflect the quality of the product nor the cleanliness of the plant. However, plate counts made at frequent intervals over extended periods of time may give the plant operator information which will allow him to predict how long he can continue to operate before he can expect a build-up of contamination in the equipment.

The desirability of standardizing microbiological techniques is one of the many problems which will eventually confront the manufacturers of frozen citrus concentrates. As might be expected in the case of such a recently developed industry, several recommendations have been made with regard to the proper techniques and media to be used in the bacteriological analysis of these products.

It is the purpose of this report, therefore, to compare the efficiencies of various plating media which have been recommended.

On the basis of experience resulting from the use of separate media for the determination of total counts and yeast and mold counts in concentrated citrus juices, it is evident that the microflora

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of these products can range from predominantly yeast to predominantly bacteria. Consequently any medium which may be selected as a standard medium for the determination of a total count in citrus products should be capable of supporting good growth of both bacteria and yeasts.

Experimental

Representative ratios of bacteria and yeasts were obtained by the use of washed suspensions of cells. For each run 10 different bacteria were isolated from frozen orange concentrate and grown on dextrose agar slants. After 24 hours incubation of 30° C. the growth was washed from the surface of these slants with 5 ml. aliquots of a sterile molar phosphate buffer (pH 7.0) and combined. A composite yeast suspension was obtained in a similar manner. These suspensions were then plated on dextrose agar and adjusted by diluting with sterile buffer so that each suspension contained approximately equal numbers of cells per ml. These suspensions were then mixed in ratios varying from all bacteria to all yeasts and appropriate dilutions of all ratios plated in duplicate on each of ten different plating media.

The composition of the plating media included in this investigation were as follows:

Lindegren Agar No. 1: 4.0 percent dextrose, 0.5 percent yeast extract, 0.35 percent proteose peptone, 0.2 percent KH_2PO_4, 0.1 percent $MgSO_4$, and 2.0 percent agar. To each liter of this medium 7 ml. of a 50 percent medium lactate solution was added.

Lindegren Agar No. 2: Similar to the above medium but modified to contain 2.0 percent dextrose and 0.1 percent KH_2PO_4 instead of the 4.0 percent and 0.2 percent respectively which were present in the original medium.

Tomato Serum Agar: 1.0 percent tryptone, 0.3 percent beef extract, 0.2 percent K_2HPO_4, 0.3 percent yeast extract, 0.5 percent dextrose, and 1.7 percent agar. Two hundred ml. of a clear tomato serum was added to each liter of medium.

Dextrose Tryptone-Yeast Extract Agar: 1.0 percent tryptone, 0.5 percent yeast extract, 0.3 percent beef extract, 0.1 percent glucose, 0.1 percent K_2HPO_4, and 1.5 percent agar.

The remaining six media (Table 1) used were prepared from dehydrated Difco media.

Results and Discussion

The results in Table 1 represent the averages of four separate experiments. Thus the averages of the five ratios

TABLE 1.
COMPARISON OF PLATING MEDIA AT DIFFERENT BACTERIA: YEAST RATIOS

Medium	pH	Bacteria: Yeast Ratios					
		4:0	3:1	2:2	1:3	0:4	Average
Tryptone-glucose-extract agar (Difco)	7.0	34.3[1]	43.5	42.0	37.8	40.3	39.6
Potato dextrose agar (Difco)	3.5	0	5.0	16.0	25.0	30.0	15.2
Lindegren yeast agar No. 2	5.8	34.0	36.8	42.5	35.5	46.3	39.0
Tomato serum agar	6.5	35.8	42.3	45.5	40.0	42.5	41.2
Sabouraud dextrose agar (Difco)	5.6	22.5	33.0	40.5	45.5	39.8	36.3
Dextrose-tryptone-yeast extract agar	7.0	40.3	41.8	40.5	43.0	42.3	41.6
Dextrose-tryptone agar (Difco)	6.7	35.3	43.0	42.5	39.3	40.3	40.1
Dextrose agar (Difco)	7.3	35.3	43.0	49.0	43.3	41.7	42.7
Nutrient agar (Difco)	6.8	37.0	32.8	31.3	38.8	37.0	35.4
Lindegren yeast agar No. 1	5.8	24.5	35.8	38.0	42.8	39.3	36.1

[1] All counts X 10^3.

which are given for each medium actually represent the averages of 20 separate observations.

The least significant mean difference between the averages of 20 observations, as determined by statistical methods, was 7.14. From an examination of this data it is evident, that, of the ten media tested, only acidified potato dextrose agar and nutrient agar yield results significantly lower than the best medium which, according to the conditions of this experiment, was Bacto-dextrose agar. Potato dextrose agar acidified to pH 3.5 finds common usages as a selective medium for the enumeration of yeasts and molds, since at this low pH it will not support bacterial growth. This medium, while not suitable as a medium for total counts, would be valuable for determining the relative proportions of bacteria and yeasts in citrus juices when used in conjunction with a medium which would grow both yeasts and bacteria.

TABLE 2.
Comparison of Dextrose Broth and a Liquid Yeast Medium with Regard to Their Abilities to Support Bacterial Growth.

Culture No.	Dextrose Broth (pH 7.3)	Lindegren Broth (pH 5.8)
M-4	73[1]	100
M-5	77	86
M-6	82	93
M-9	97	100
M-10	68	90
M-11	73	95
M-13	70	93
M-14	97	98
M-17	71	94
M-20	82	82
M-21	91	86
M-22	65	91
M-23	90	90
M-24	69	90
M-25	84	99
M-26	70	98
M-27	91	95
M-28	71	95
M-29	68	98
M-30	86	97
M-32	69	95
M-33	75	95
M-34	56	76
M-36	73	95
M-42	89	95
M-43	69	67
M-56	75	95
M-65	100	100
M-66	60	88
M-73	59	66
M-75	99	95
M-92	62	72
M-99	1	71
Average	77	90

[1] Figure represents percentage of light transmitted through culture based on 100 percent light transmission through an uninoculated tube of the same medium.

On the other hand, media having pH values considerably below the optimum for most bacteria have gained wide usage in the frozen concentrate industry. Sabouraud dextrose agar and Lindegren's yeast medium, with pH values of 5.6 and 5.8 respectively, are recommended for the isolation and cultivation of yeasts and molds. Although the addition of an inhibitory substance such as copper sulfate is required to completely suppress bacterial growth, the medium alone will inhibit many bacteria to varying degrees.

This inability of a medium of this type to support good growth of many species of bacteria is clearly illustrated in Table 2. Thirty-three bacterial isolates originating from frozen orange concentrate were grown in broth counterparts of dextrose agar and the modified Lindegren agar which were included in Table 1. Following a 48 hours incubation period at 30° C. the degree of growth in these tubes was measured turbidimetrically in a Fisher electrophotometer. The values given in Table 2 represent the percentage of light transmitted by the cultures based on 100 percent light transmission through the uninoculated medium. The superiority of the dextrose broth over the yeast medium is shown by the values of

TABLE 3.
COMPARISON OF PLATING MEDIA AT DIFFERENT BACTERIA: YEAST RATIOS

Medium	pH	Bacteria: Yeast Ratios 4:0	3:1	2:2	1:3	0:4	Averages
Dextrose agar (Difco)	7.3	580[1]	330	390	290	40	326
Dextrose-tryptone agar (Difco)	6.7	560	420	440	270	22	342
Tryptone-glucose extract agar (Difco)	7.0	590	610	420	250	40	382
Dextrose-tryptone yeast extract agar	7.0	540	560	500	260	23	417
Sabouraud dextrose agar (Difco)	5.6	4.1	14	36	17	40	22.2
Lindegren yeast agar No. 1	5.8	4.3	15	29	15	33	19.3
Malt agar (Difco)	3.5	0	0.6	1.8	2.5	3.4	1.7
Potato dextrose agar (Difco)	3.5	0	1.0	1.8	3.0	3.6	1.9

[1] All counts X 10^3.

77 and 90 respectively for the averages of the 33 cultures. Seventeen of the total number of cultures gave turbidity readings of 95 or higher in the yeast medium indicating very slight growth of these organisms.

The data presented in Table 3 represent a preliminary run which was not included in Table 1, since at the time of this particular experiment, several of the media listed in Table 1 had not been introduced into the frozen concentrate industry. The results of this run are inincluded at this point, however, to show the type of results which might be expected if the greater majority of the bacteria present in a juice were strains which did not grow well in a low-pH medium. During the isolation of the bacteria used in this run no attempt was made to isolate organisms of this type.

Comparable results were obtained with the four media whose pH values ranged from 6.7 to 7.3. Sabouraud dextrose agar and the Lindegren yeast medium, however, gave satisfactory results only when a suspension composed entirely of yeasts was plated. Malt agar and potato dextrose agar, both acidified to a pH of 3.5, were unsatisfactory at all ratios in this particular instance.

Summary

From these results it is apparent that under normal conditions any of the media included in Table 1, with the exception of acidified potato dextrose agar and possibly nutrient agar, could be employed satisfactorily as a medium for obtaining a total microorganism count in citrus juices. However, the limitations of any of these media should be clearly understood. No single medium will support the growth of all types of microorganims equally well, and the possibility always exists that abnormally high counts resulting from a build-up of contamination in the plant equipment might not be reflected in the counts obtained during routine examinations if a single plating medium is used.