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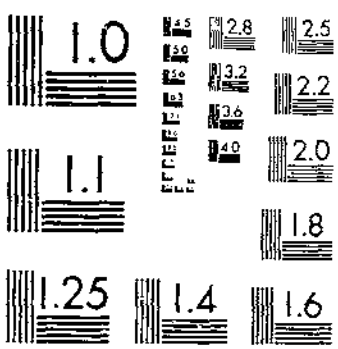

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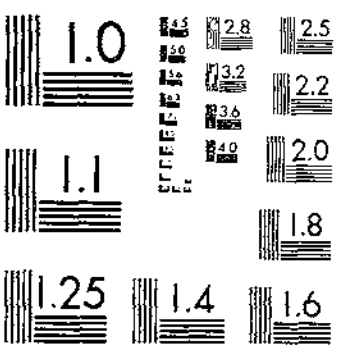

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STICKINESS AND SPOTTING OF SHELLED GREEN LIMA BEANS[1]

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INTRODUCTION

Fresh lima beans develop two characteristic and serious troubles when held or shipped after being shelled. One of these is a slimy, sticky condition of the surface, which causes the beans to stick to the fingers when touched; the other is a superficial spotting.

The spots are usually small, 1 to 3 mm in diameter, with a rather indefinite margin (fig. 1). They are usually brown, but if the beans are held under very humid conditions the color may become olivaceous. In the early stages of the trouble only the testa is affected, but in later stages brown spots on the cotyledons are evident upon the removal of the testa.

Many inquiries have been received by the United States Department of Agriculture in regard to the above troubles, especially stickiness, and the following experiments were undertaken to determine the nature of the diseases and to meet the demand for a remedy.

MATERIALS AND METHODS

Except where otherwise stated the beans used in the present experiments were grown at Arlington Experiment Farm, Arlington, Va., and were freshly picked. The beans from Florida, Cuba, and California were purchased in the pod on the Washington, D. C., market.

[1] Submitted for publication June 2, 1937.

[2] Earlier unreported studies on the present problem were made by R. C. Wright and T. M. Whiteman, of the Division of Fruit and Vegetable Crops and Diseases, who found that the two diseases could be largely controlled by treating the shelled beans with different concentrations of ethyl alcohol.

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Freshly shelled beans were used in all experiments. Unless otherwise stated the beans were stored in fruit jars. The jars were closed with the usual tops but with strips of cardboard beneath the tops so as to allow a slight exchange of air.

The fungus inoculations were made by immersing the beans in a suspension of the spores in sterile water. In the bacterial inoculations 24-hour beef-broth cultures were diluted 1 to 10 with sterile water, and the beans immersed in the solution. The control lots of beans were immersed in sterile water or in similarly diluted sterile broth. The beans were allowed to dry before storing.

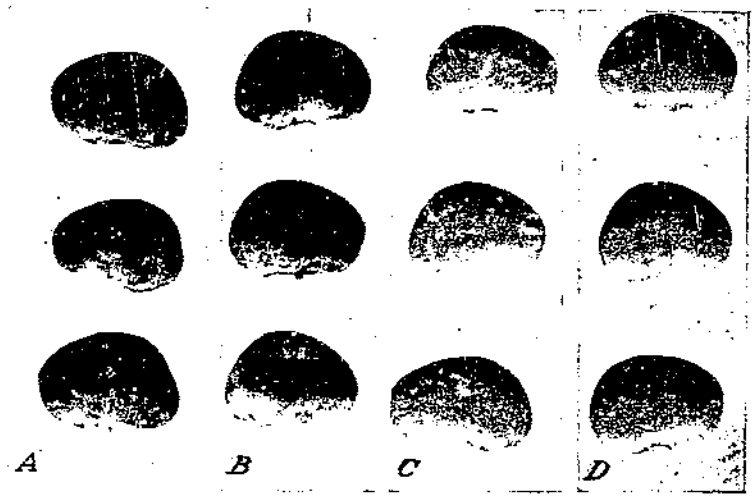

FIGURE 1.—Spotting of Henderson Bush lima beans as affected by exposure to carbon dioxide. The beans were picked September 25, 1931: *A*, 50° F. storage; *B*, control refrigerator, with temperature dropping from an initial 77° to a final 41° and averaging 48°; *C*, refrigerator, with temperature dropping from 75° to 47°, averaging 53°, and with carbon dioxide dropping from 31 percent to 15 percent and averaging 24 percent; *D*, refrigerator, with temperature dropping from 75° to 48°, averaging 56°, and with carbon dioxide dropping from 51 percent to 38 percent, averaging 41 percent. Photographed after 2 days under experimental conditions; subsequent storage of 4 days at 40° and 1 day at 70°.

In determining the results of the various treatments the degree of stickiness was estimated by the extent to which the beans would stick to the fingers and by the jarring required to dislodge them. As a means of comparing the different lots, the stickiness was rated as percent, with 100 as the worst condition possible.

The effect of the various treatments upon spotting was determined on the basis of the average number of spots per bean. This number was arrived at by estimation, with occasional counts as a check on the accuracy of the estimate.

ISOLATIONS AND INOCULATIONS

BACTERIA

Cultures from the sticky surfaces of shelled lima beans from California, Virginia, and Florida gave an abundant development of bacteria. Pure cultures were obtained of the organisms that were present in greatest numbers and these were tested by inoculations on other shelled beans. The results of these inoculations are shown in table 1. The cultures were named according to the place of origin of the beans.

TABLE 1. *Inoculation experiments on green lima beans*

Variety and origin of beans	Date	Previous treatment	Inoculum	Storage		Condition after storage			
				Period	Temperature	Spots per bean		Stickiness	
						Inoculated	Control	Inoculated	Control
				Days	*F.*	*Number*	*Number*	*Percent*	*Percent*
Fordhook Calif	Nov. 9 1934	None	Bacterium Va. 1	5	40	25	25	50	35
				1	70				
Do	do	do	Bacterium Va. 2	5	40	45	25	60	35
				1	70				
Do	do	do	Bacterium Va. 3	5	40	30	25	60	35
				1	70				
Do	do	do	Bacterium Va. 4	5	40	5	25	30	35
				1	70				
Do	do	do	Bacterium Va. 5	5	40	45	25	50	35
				1	70				
Do	Nov. 16 1934	do	Bacterium Va. 2	1	70	15	20	60	40
				2	50				
Do	do	do	Bacterium Va. 3	1	70	20	20	40	40
				2	50				
Fordhook Fla	Dec. 5, 1934	30 percent alcohol, 1 minute	do	6	50	0	0	60	13
Fordhook Calif	Dec. 13, 1934	do	do	7	50	0	0	85	30
Do	do	do	Cladosporium	11	50	70	0	40	60
Fordhook Fla	Mar. 28, 1935	do	Bacterium Calif. 1	8	50	0	0	30	10
Do	do	do	Bacterium Calif. 2	8	50	0	0	45	10
Do	do	do	Bacterium Va. 1	8	50	0	0	45	10
Do	do	do	Bacterium Va. 2	8	50	0	0	30	10
Do	do	do	Bacterium Va. 3	8	50	0	0	25	10
Do	do	do	Bacterium Va. 4	8	50	0	0	10	10
Do	do	do	Bacterium Va. 5	8	50	0	0	4	10
Do	do	do	Bacterium Va. 6	8	50	0	0	35	10
Do	do	do	Bacterium Va. 7	8	50	0	0	45	10
Do	do	do	Bacterium Va. 8	8	50	0	0	10	10
Do	do	do	Bacterium Va. 9	8	50	0	0	55	10
Do	do	do	Bacterium Fla. 1	8	50	0	0	6	10
Do	do	do	Bacterium Fla. 2	8	50	0	0	50	10
Do	do	do	Bacterium Fla. 3	8	50	0	0	50	10
Do	do	do	Bacterium Fla. 4	8	50	0	0	50	10
Do	Apr. 18, 1935	Tap water	Cladosporium	7	50	80	0	4	30
Do	do	do	Bacterium Calif. 2	7	50	0	0	60	30
Do	do	do	Bacterium Va. 9	7	50	0	0	60	30
Do	do	do	Bacterium Fla. 2	7	50	0	0	55	30
Do	Apr. 26, 1935	do	Cladosporium	6	50	75	5	50	35
Fordhook Va	Sept. 23, 1935	30 percent alcohol, 1 minute	Bacterium Va. 9	5	50	0	0	33	3
Do	do	do	Cladosporium	7	50	50	0	38	35

In a few instances the beans inoculated with bacteria developed more spots than the controls and in others the reverse condition held, but in general there was no indication that the bacteria had any effect upon spotting.

On the other hand, with the exception of one of the four cultures from Florida beans and three of the nine cultures from Virginia beans, the bacterial inoculations caused a decided increase in stickiness. This increase was 40 to 100 percent with beans that had received no previous disinfecting treatment and 150 to 1,000 percent with beans that had been disinfected with alcohol prior to inoculation.

There was little contrast in the results obtained with bacteria from California, Florida, or Virginia beans, indicating that organisms capable of producing stickiness have a wide distribution. Calif. 2, Fla. 2, and Va. 9 were three of the particularly active organisms and were selected for use in most of the physiological and control experiments.

FUNGI

When spotted beans were examined under the microscope, mycelium was plainly evident. Isolations from the affected tissue uniformly gave cultures of *Cladosporium*. The results of inoculations with this fungus are shown in table 1.

In two of the *Cladosporium* inoculations stickiness was decidedly decreased and in the other two it was increased. In these and other experiments there was some indication that an abundant *Cladosporium* growth tended to decrease stickiness, possibly because of decreasing the surface moisture available for the bacteria.

The effect of the *Cladosporium* inoculations upon the occurrence of spotting was most pronounced. In one test the inoculation increased the average number of spots per bean from 5 to 75 and in the three other tests from zero to 50, 70, and 80. The original *Cladosporium* was reisolated readily from the inoculated beans. The results seem to leave no doubt as to the part played by *Cladosporium* in the production of lima bean spotting.

EFFECT OF HUMIDITY

Experiments were undertaken to determine the effect of humidity under storage conditions. The results shown in figure 2 give conclusive evidence that both spotting and stickiness were highly responsive to moisture conditions. A drop in the relative humidity of the storage atmosphere from 96 to 73 percent resulted in a decrease in stickiness ranging from 50 to 73 percent and a decrease in the number of spots of 60 to 84 percent. Likewise, a drop in humidity from 96 to 88 percent gave a decrease of 18 to 37 percent in stickiness and of 30 to 40 percent in number of spots.

The beans became badly shriveled when held in an atmosphere showing 73 percent relative humidity.

In the more humid atmospheres the spots were olivaceous in color; under drier conditions they were brown.

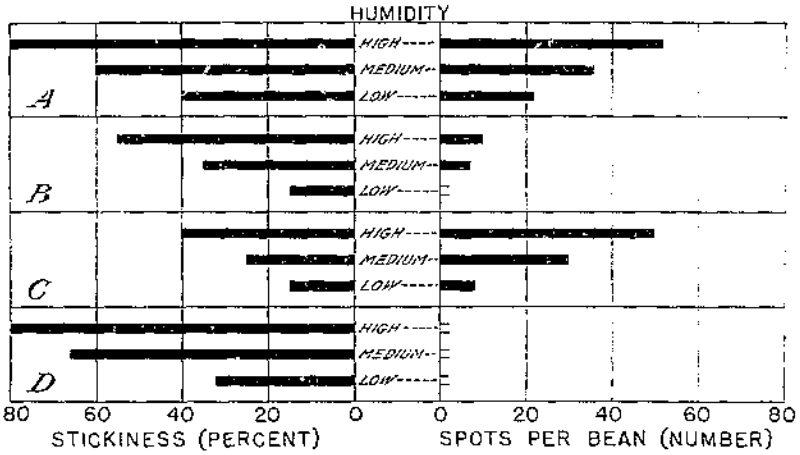

FIGURE 2.—Effect of humidity upon the development of stickiness and spotting of shelled green lima beans. All lots were stored at 50° F. in open containers in large metal boxes. The high-, medium-, and low-humidity boxes had relative humidities of approximately 96, 88, and 73 percent, respectively. *A*, King of the Garden lima beans, September 11, 1935; inoculated with *Cladosporium*; record after 5 days' storage. *B*, King of the Garden lima beans September 11, 1935; washed in 30-percent alcohol 1 minute, rinsed in water, then inoculated with bacterium Fla. 2; record after 5 days' storage. *C*, Fordhook lima beans, September 23, 1935; washed in 30-percent alcohol 1 minute, rinsed in water, then inoculated with *Cladosporium*; record after 7 days' storage. *D*, Fordhook lima beans, September 23, 1935; washed in 30-percent alcohol 1 minute, rinsed in water, and inoculated with bacterium Fla. 2; record after 7 days' storage.

EFFECT OF TEMPERATURE ON STICKINESS

The effect of temperature upon the development of stickiness is shown in figures 3 to 7, inclusive. The beans were neither washed nor disinfected, and, with the exception of those reported in figure 3, they were not inoculated. The development at 77° F. was more rapid than at 68°, but at either of these temperatures uninoculated Fordhook and King of the Garden beans had become quite sticky by the end of 2 days, and Henderson Bush and Dreer Bush lima beans by the end of 4 days (figs. 4–7). The development at 59° was 24 to 36 hours later than that at 68°, the development at 50° usually more than 2 days later than at 59°, and that at 41° more than 4 days later than at 50°. At 41° the beans usually remained practically free from stickiness for 1 week or more, and at 32° for 2 weeks. The development at 68° was usually two to two and one-half times as rapid as at 50°.

The development of stickiness on inoculated beans was more rapid than on the uninoculated ones (fig. 3), but there was the same general temperature response. The results reported in figure 3 would indicate that there was no significant difference in the temperature response of bacteria from different sections of the country, but the beans of this experiment were not disinfected prior to inoculation, and therefore carried their chance supply of bacteria as well as those added by inoculation.

The effect of temperature on the growth rate of bacterium Fla. 2 in Petri plate cultures is shown in figure 8. At the end of 4 days the average diameter of the colonies at 77°, 68°, 59°, and 50° F. was 16, 12.5, 5.2, and 3.5 mm, respectively, whereas no growth was evident at 41° at that time or 2 days later. At the end of 11 days the average diameter of the colonies at 59°, 50°, and 41° was 11.1, 7.5, and 3.0 mm, respectively, but no growth was evident at 32°.

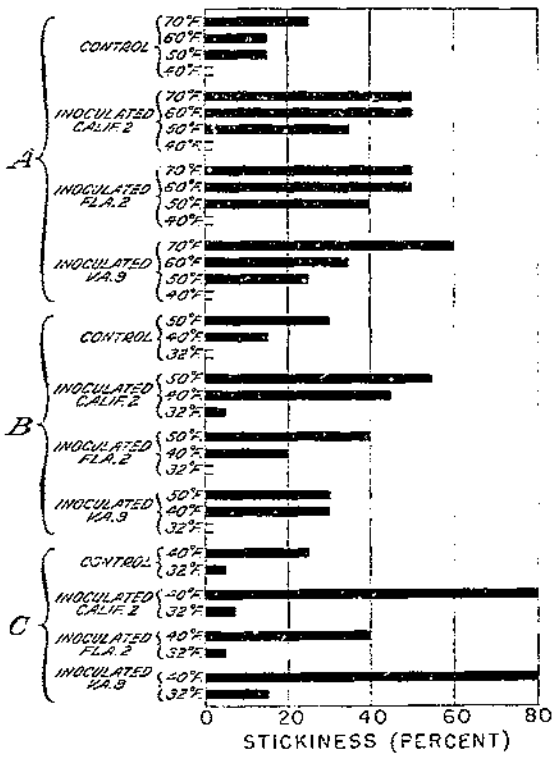

FIGURE 3.—Inoculation and temperature studies on the production of stickiness of shelled Florida Fordhook Lima beans. *A*, At the end of 4 days; *B*, at the end of 8 days; and *C*, at the end of 14 days.

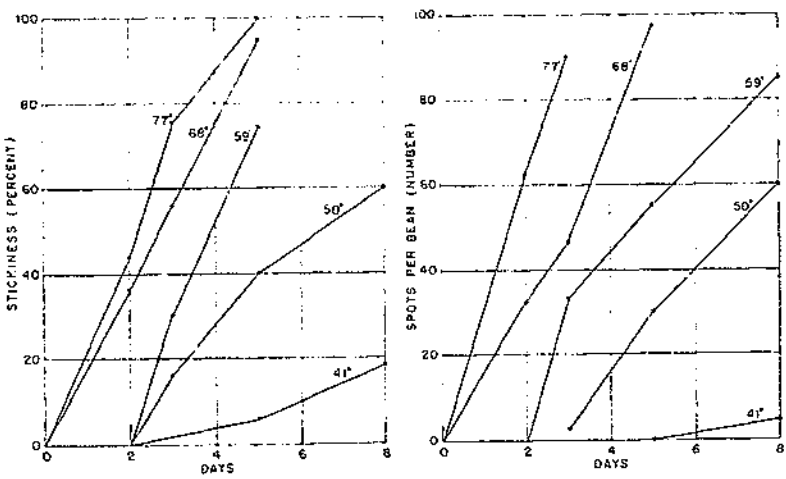

FIGURE 4.—Effect of temperature on the development of stickiness and spotting of King of the Garden lima beans, picked September 11, 1935.

The above experiments indicate the great value of low temperature in preventing the development of stickiness. It is evident that temperatures of 50° F. or higher cannot be relied upon to hold stickiness

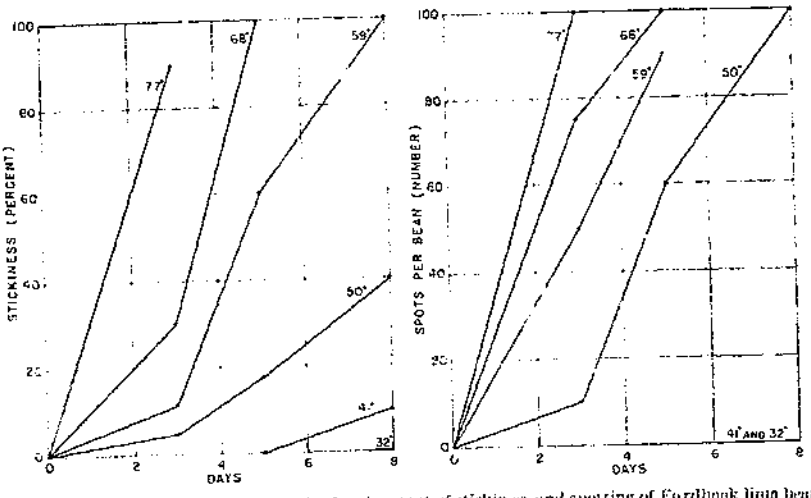

FIGURE 5.—Effect of temperature upon the development of stickiness and spotting of Fordhook lima beans, picked September 25, 1935.

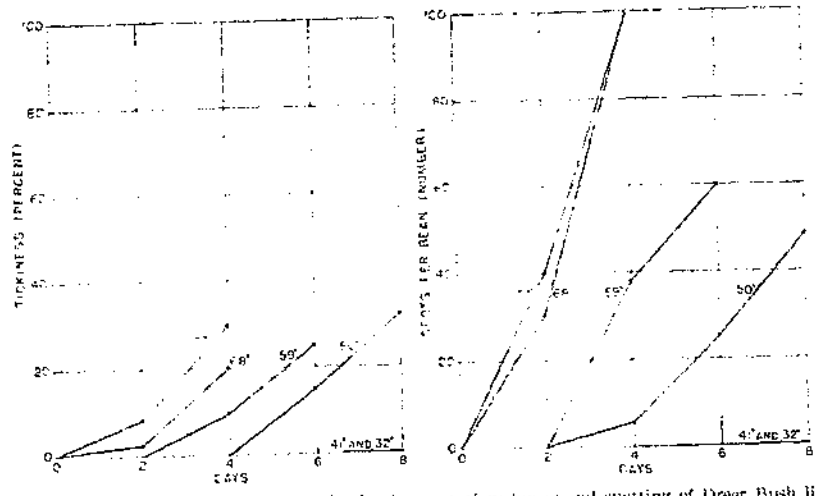

FIGURE 6.—Effect of temperature upon the development of stickiness and spotting of Dreer Bush lima beans, picked October 5, 1935. The beans were stored in paper bags.

in check over any considerable period, whereas a temperature of 41° gives complete control for a period of 6 to 8 days and a temperature of 32° complete control for 10 to 14 days.

EFFECT OF TEMPERATURE ON SPOTTING

The effect of temperature upon the development of spotting is shown in figures 4 to 7, inclusive, and its effect upon the growth rate of *Cladosporium* is shown in figure 9.

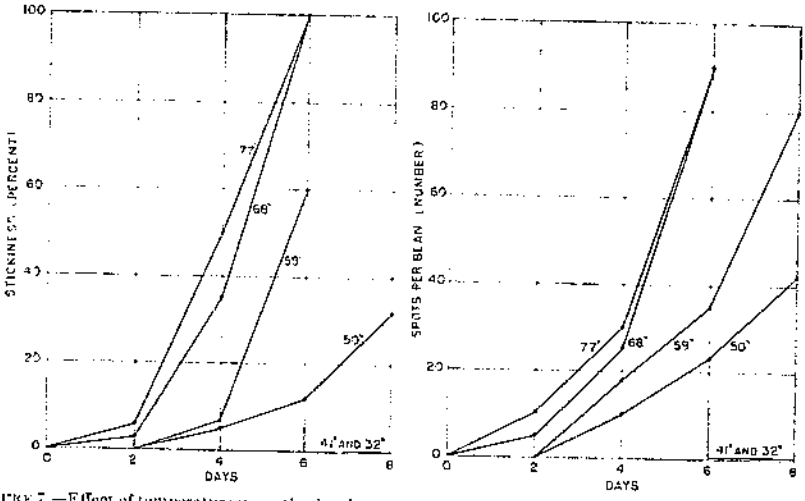

FIGURE 7.—Effect of temperature upon the development of stickiness and spotting of Henderson Bush lima beans, picked October 5, 1935.

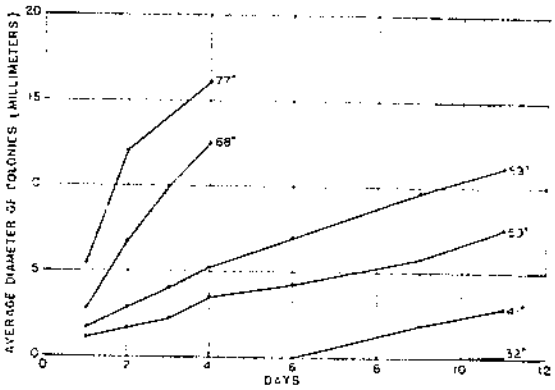

FIGURE 8.—Temperature-growth curves for bacterium Fla. 2, as shown in the diameter of the colonies. Growth on beef agar of pH 7.

With the increase in temperature from 50° to 68° F., the rate of growth and the development of spotting became two to two and one-half times as rapid; above 68° the increase in spotting was not so rapid, and on Thaxter's agar the fungus grew but little better at 77° than at 59°; below 50° the development of spotting was greatly delayed. The contrast between 41° and 50° was nearly as great as that between 50° and 68°, and at 32° the growth of the fungus was almost completely inhibited.

EFFECT OF EXPOSURE TO CARBON DIOXIDE ON STICKINESS AND SPOTTING

Experiments were carried out to determine the effect of exposure to carbon dioxide upon the development of stickiness and spotting. In the experiments reported in figures 10 and 11 the beans were held at constant temperatures and in atmospheres kept uniform by continuous renewal. Approximately six changes of air were made each 24 hours. Some of the beans were held in atmospheres containing 35 percent of carbon dioxide, and others at the same temperature in air that was practically free from carbon dioxide. The atmospheres were prepared by mixing carbon dioxide with air by means of flowmeters.

Other carbon dioxide experiments were made in pony refrigerators as described in an earlier publication (3).[4] The modified atmospheres

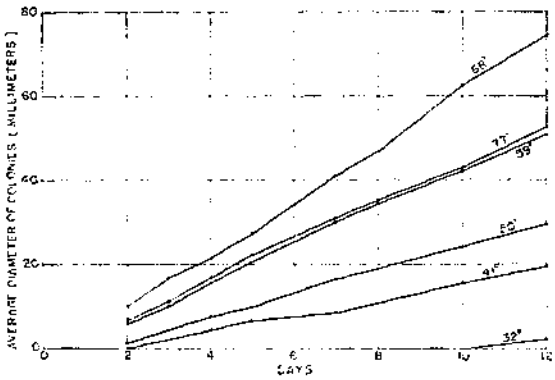

FIGURE 9.—Temperature-growth curves for *Cladosporium* from spotted lima beans. Growth on Thaxter's agar.

were obtained by supplementing the ordinary icing with the addition of a small quantity of solid carbon dioxide. The temperature and carbon dioxide conditions of such an experiment are shown in figure 12, and the effects of the treatments upon stickiness and spotting are reported in figure 13. The experimental conditions were continued for 51 hours, then all lots were moved to moist chambers at 50° F.

The treatments for lot *C* were similar to those for *A* and *B*. The temperatures in the three refrigerators were practically identical and averaged approximately 48° F. The maximum carbon dioxide in *C*, 3, was 66, the minimum 54, and the average 60 percent. The carbon dioxide in *C*, 4, ranged from a high of 36 percent to a low of 16, and averaged 25. After 2 days under the experimental conditions all lots were moved to moist chambers at 50°.

The development of stickiness on the beans held in carbon dioxide at a particular temperature was approximately the same as that on the controls held at a temperature 18° lower (figs. 10 and 11). For instance, the results at 77° F. with carbon dioxide were similar to those at 59° without carbon dioxide, and those at 50° with carbon dioxide were similar to those at 32° without carbon dioxide.

[4] Italic numbers in parentheses refer to Literature Cited, p. 24.

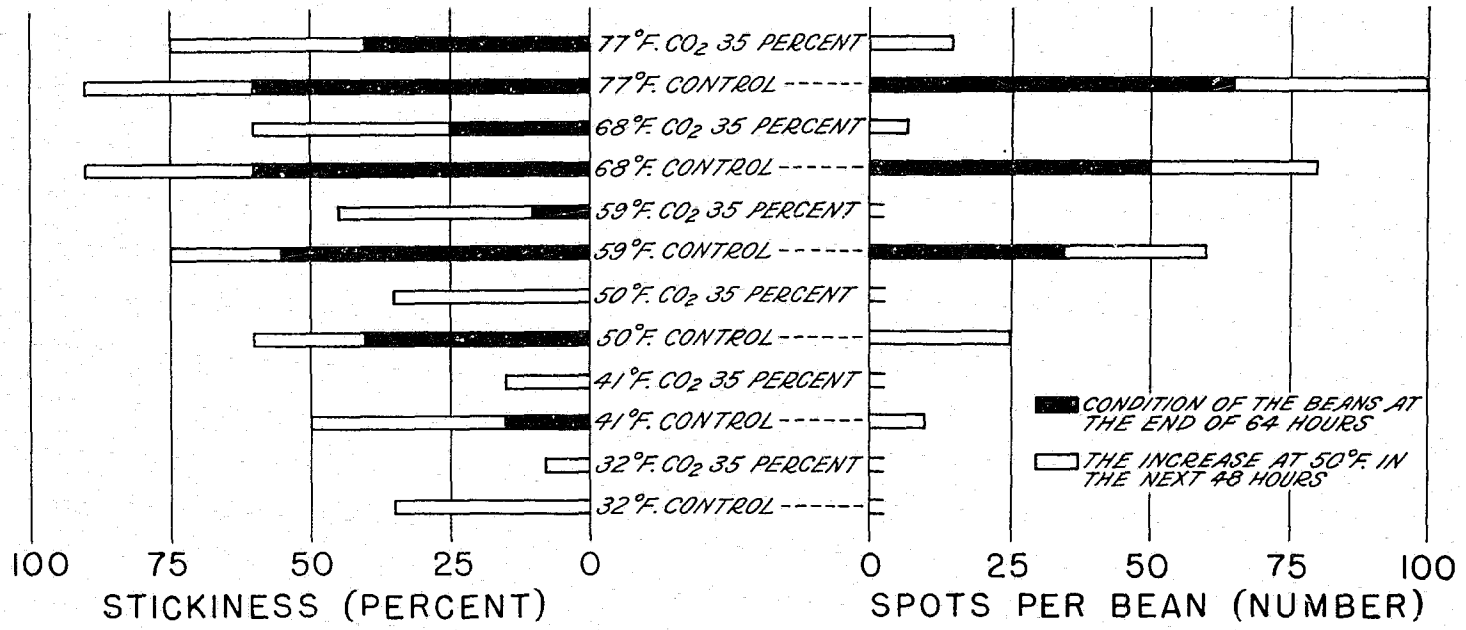

FIGURE 10.—Effect of exposure to carbon dioxide upon development of stickiness and spotting in King of the Garden green lima beans, picked September 11, 1935. The beans were held at the listed temperatures in controlled atmospheres for 64 hours and then were removed to moist chambers at 50° F.

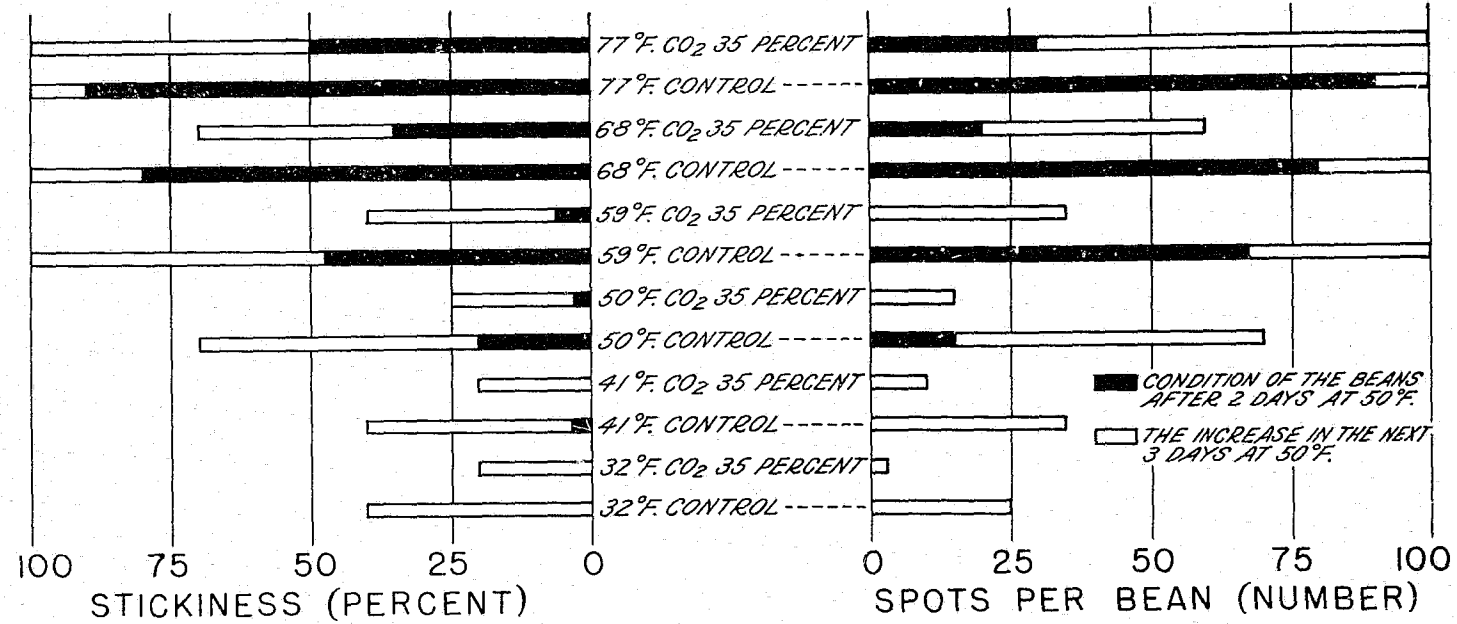

FIGURE 11.—Effect of exposure to carbon dioxide upon development of stickiness and spotting in Dreer Bush lima beans, picked September 26, 1935. The beans were held at the listed temperatures in controlled atmospheres for 42 hours and then were removed to moist chambers at 50° F.

In the pony-refrigerator experiments, as shown in figure 13, *A* and *B*, exposure of the beans to 25 percent or more of carbon dioxide resulted in a delay in the development of stickiness that was decidedly longer than the period of treatment, whereas in *C* the delay was not so long as the period of treatment. The results would indicate that the bacteria that produce stickiness were greatly inhibited by the carbon dioxide treatments. Twenty-five percent of carbon dioxide gave nearly as great inhibition as 50 or 60 percent.

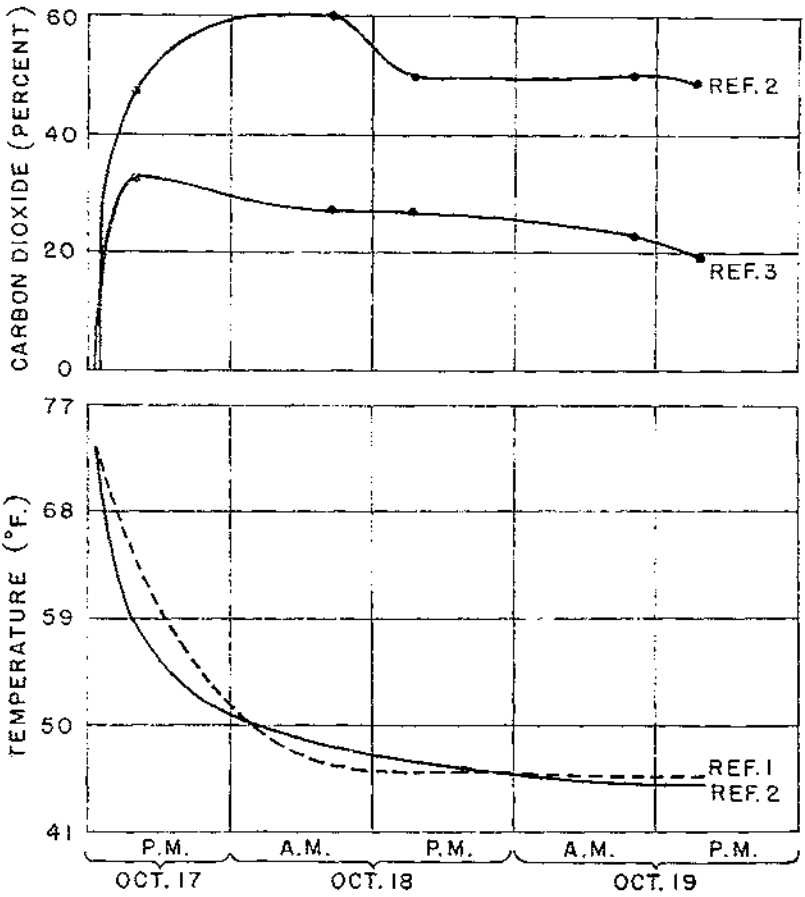

FIGURE 12.—Carbon dioxide and temperature curves in an experiment with Wood Prolific and New Philadelphia lima beans, picked October 17 to 19, 1934. Ref. 1, control refrigerator; ref. 2 and ref. 3, carbon dioxide, as shown in the upper part of the figure. (The thermograph in ref. 3 failed to record, but the temperature conditions would have been similar to those in the other two refrigerators.)

In these experiments records were kept on spotting as well as on stickiness (figs. 10, 11, and 13), and it was found that carbon dioxide had a greater inhibiting effect upon spotting than upon the stickiness. In all the tests reported in figures 10 and 11 the inhibition from exposure to carbon dioxide was as great as that resulting from an 18° drop in temperature and in many cases equal to that of a 27° drop.

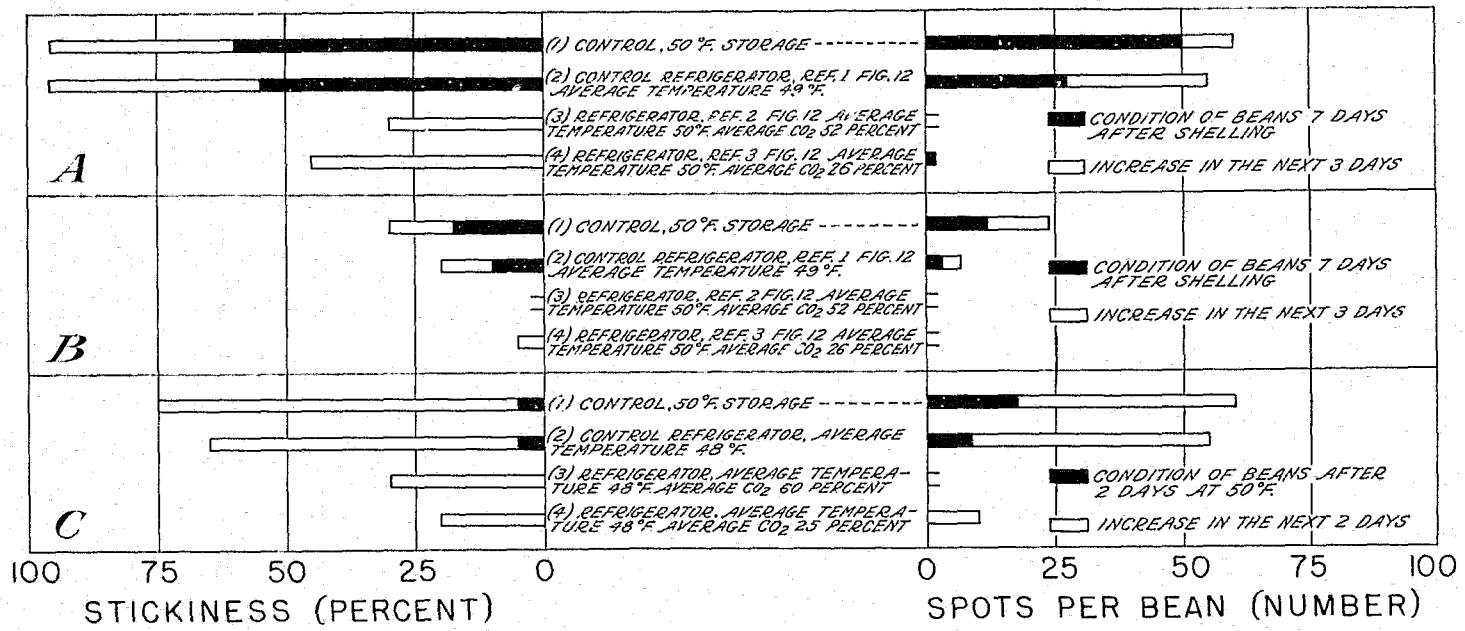

FIGURE 13.—Effect of exposure to carbon dioxide upon development of stickiness and spotting in green lima beans held in pony refrigerators; *A*, New Philadelphia, and *B*, Wood Prolific beans, picked October 17, 1934; *C*, lima beans purchased on the Washington, D. C., market, October 25, 1934.

In the pony-refrigerator experiments reported in figure 13, *A* and *B*, the period of delay resulting from the carbon dioxide treatment was two to three times as great as the period of treatment; in those reported in *C* the delay was fully as great as the period of treatment. These results indicate that carbon dioxide not only may completely inhibit spotting but also produce conditions unfavorable for its later development. Twenty-five percent of carbon dioxide gave nearly as great inhibition of spotting as 50- or 60-percent concentrations.

Three earlier carbon dioxide experiments were made with conditions and results similar to those reported in figures 12 and 13, but the records were not taken in a form that could be tabulated. However, the very effective control of spotting in one of these tests is shown in figure 1.

EFFECT OF EXPOSURE TO CARBON DIOXIDE ON FLAVOR AND QUALITY

A cooking test was made on the beans of the experiments reported above, and those from the carbon dioxide lots were found to be fully as good as those from the controls. This is in agreement with an earlier report (*2*).

Miller and Dowd (*5*) found that carbon dioxide had a favorable effect upon the retention of sugar in lima beans. Beans held at 77° F. in an atmosphere containing 42 percent of carbon dioxide retained their sugar almost as well as control lots at 59°. At lower temperatures the effect of the carbon dioxide was less pronounced.

EFFECT OF DISINFECTING WASHES

Various disinfecting washes were tested in a study of possible methods of control and as a means of obtaining further information as to the nature of the two diseases. If the inoculation experiments previously reported (p. 3) were not sufficiently convincing, the results of the disinfecting tests leave no doubt as to their infectious nature.

The records on spotting and stickiness were taken as previously described (p. 2). The record on injury was taken as percent. Where the injury is reported as less than 5 percent it was usually in the nature of a slight bleaching of the beans. Where it is reported as 5 percent or more there was usually a browning of the testa or other rather conspicuous injury. The least mature beans were most susceptible to injury. All of the treatments were given at room temperature. Some of the tests were made on shelled beans and others on the pods before shelling.

SHELLED-BEAN TREATMENTS

The results of the disinfecting treatments on shelled beans are reported in table 2. In three of the tests one control lot of shelled beans was washed in tap water and another left without washing. The washing had little if any inhibiting effect upon the development of stickiness but caused a decided reduction in spotting.

The only disinfecting treatment found that showed promise of being commercially practicable was that with ethyl alcohol (nondenatured). The usual procedure was to immerse the beans in a 30-percent alcohol solution for 1 minute, then remove them and allow several minutes' free exposure before storing. In addition to its dis-

infecting properties the rapid evaporation of the alcohol greatly facilitated the drying of the beans.

In all of the 12 tests, the alcohol treatment eliminated spotting for the storage period of 5 to 9 days and greatly delayed the development of stickiness. In some cases a particular degree of stickiness was attained 2 days later on the treated than on the control lots, and in other cases 4 to 6 days later. With other lots (7 and 12, table 2) stickiness appeared to be permanently eliminated. In records taken 6 to 8 days after treatment the stickiness of the treated beans averaged more than 70 percent below that of the control lots.

TABLE 2.—*Disinfection experiments on shelled green lima beans*

Lot	Storage		Treatment before storage	Condition after storage		
	Period	Temperature		Spots per bean	Stickiness	Injury
	Days	*°F.*		*Number*	*Percent*	*Percent*
1	...	...	Sterile water, 1 minute	25	35	0
			1 percent HCl, 1 minute	2	5	85
			1 percent HCl, 10 seconds	10	5	60
			0.5 percent HCl, 1 minute	4	1	30
			0.25 percent HCl, 10 minutes	10	5	60
2	...	...	Sterile water, 1 minute	50	60	0
			Dilute beef broth, 1 minute	50	60	0
			0.25 percent HCl, 1 minute	5	5	10
			10 percent $C_2H_4O_2$, 1 minute	0	0	25
			10 percent $C_2H_4O_2$, 1 minute	0	10	35
			30 percent C_2H_5OH, 1 minute	0	0	5
			0.5 percent $CuSO_4$, 1 minute	6	10	35
3	7	50	None	62	58	0
			Washed in running tap water, 5 minutes	21	25	0
			30 percent C_2H_5OH, 1 minute	0	28	0
4	7	50	None	5	30	0
			Washed in running tap water, 45 minutes	0	30	0
			30 percent C_2H_5OH, 1 minute, rinsed in water	0	10	1
5	8	47–50	None	30	60	0
			30 percent C_2H_5OH, 1 minute	0	16	0
6	7	45 50	None	25	60	0
			0.5 percent HCl, 1 minute	16	10	0
			30 percent C_2H_5OH, 1 minute	0	20	0
			30 percent C_2H_5OH, 1 minute, then in dilute beef broth, 1 minute	0	13	0
7	8	50	None	96	30	0
			Washed in running tap water, 10 minutes	40	50	0
			30 percent C_2H_5OH, 1 minute	0	0	1
8	7	50	None	56	65	0
			30 percent C_2H_5OH, 1 minute	0	35	0
			30 percent C_2H_5OH+0.02 percent HCl, 1 minute	0	5	0
			0.02 percent HCl, 1 minute	40	45	0
			1 percent ClH_2O, 1 minute	0	3	0
			1 percent ClH_2O, 2 minutes	0	3	3
			0.5 percent ClH_2O, 2 minutes	0	3	1
9	5	50	None	18	10	0
			30 percent C_2H_5OH, 1 minute	0	3	2
			1 g $Na_2H_2O_2$ to each pint container	80	30	3
			0.5 g $NaHSO_3$ to each pint container	80	30	3
			0.2 g $NaHSO_3$ to each pint container	90	35	3
10	6	50	None	80	50	0
			30 percent C_2H_5OH, 1 minute	0	10	2
			30 percent C_2H_5OH+0.05 percent HCl, 1 minute	0	5	2
			30 percent C_2H_5OH+0.02 percent HCl, 1 minute	0	5	2
			0.5 percent ClH_2O+0.02 percent HCl, 1 minute	0	2	2
11	7	50	None	80	100	0
			30 percent C_2H_5OH, 1 minute	0	10	3
			15 percent C_2H_5OH, 1 minute	0	40	3
			15 percent C_2H_5OH+0.25 percent ClH_2O, 1 minute	0	0	3
			0.25 percent ClH_2O, 1 minute	0	0	2
			0.5 percent ClH_2O, 1 minute	0	0	2

[1] Lots 1 to 7, inclusive, were Fordhook beans from Florida or California; lots 8 and 9, Fordhook from Virginia; lots 10 and 11, Dreers Bush lima from Virginia; lot 12, Henderson Bush lima from Virginia; and lot 13, Burpees Improved Bush lima from Virginia.

[2] Stored 5 days at 40° F. and then 1 day at 70°.

[3] Stored 1 day at 70° F. and then 4 days at 50°.

TABLE 2.—*Disinfection experiments on shelled green lima beans*—Continued

Lot	Storage		Treatment before storage	Condition after storage		
	Period	Temperature		Spots per bean	Stickiness	Injury
	Days	*F.*		*Number*	*Percent*	*Percent*
12	7	50	None	10	35	
			30 percent C_2H_5OH, 1 minute	0	0	0
			30 percent C_2H_5OH+0.05 percent HCl, 1 minute	0	0	0
			30 percent C_2H_5OH+0.02 percent HCl, 1 minute	0	0	0
			0.5 percent CH_2O+0.02 percent HCl, 1 minute	0	0	0
13	6	50	None	77	60	0
			30 percent C_2H_5OH, 1 minute	0	13	
			0.5 percent CH_2O, 1 minute	0	0	
			0.25 percent CH_2O, 1 minute	0	0	3
			0.25 percent CH_2O, 1 minute, in moist chamber 3 minutes	0	0	3
			0.25 percent CH_2O, 3 minutes	0	0	4

In one of the experiments, 15-percent alcohol was used in comparison with 30-percent alcohol (lot 11, table 2). It gave complete control of spotting, but much poorer control of stickiness than the 30-percent.

The alcohol treatments had a slight bleaching effect. The treated beans had less green color and less gloss than the untreated ones, but the condition was not usually noticeable except upon comparison with other lots.

Acidified alcohol was used in three of the experiments (lots 8, 10, and 12, table 2). Adding 0.02 or 0.05 percent of hydrochloric acid to the 30-percent alcohol decidedly increased its effectiveness in the control of stickiness.

It was not found possible to secure satisfactory control of stickiness and spotting with acids alone, without injury to the beans. Ten percent acetic, 10 percent lactic, and 1, 0.5, and 0.25 percent hydrochloric acid gave decided reduction in stickiness and a significant reduction in spotting when used as washes but caused sufficient injury to bar them from practical consideration.

A 0.5-percent solution of copper sulphate was used in one test and gave fair control but with severe injury. Sodium bisulphite was used in one set of experiments (lot 9, table 2). The material was wrapped in cotton to prevent contact with the beans and placed in the bottom of the jars in which the beans were stored. Using 1.0, 0.5, or 0.2 g to each pint of beans decidedly increased the development of both stickiness and spotting.

Aside from alcohol, formaldehyde made the best showing from the standpoint of disease control. Holding the beans in a 1.0-, 0.5-, or 0.25-percent solution for 1 minute gave complete control of spotting in every case and as good control of stickiness as a similar treatment with 30-percent alcohol. The results were also satisfactory from the standpoint of injury. The odor of formaldehyde soon disappeared after treatment, but chemical tests showed that the beans actually retained an appreciable amount of the material. Beans that were washed in a 0.25-percent solution gave a positive test 1 day after treatment, and beans that were washed in a 0.5-percent solution gave a positive test after washing and also after cooking. The chemical tests were made by Rimini's method as modified by Schryver and

described by Plimmer *(6)*. Tests on material having a known concentration of formaldehyde showed that the method was sensitive to 1 part in 1,000,000 and the reactions with the beans indicated that several times that amount was probably present.

The fact that formaldehyde fails to completely evaporate from the beans makes its use questionable and possibly dangerous from the standpoint of public health and leaves ethyl alcohol as the only material tested that has shown practical possibilities.

POD TREATMENTS

Since stickiness and spotting cause no trouble on unshelled beans, it seemed probable that the pods carried the causal organisms and that they were lodged on the beans in the process of shelling. If this was true it was thought probable that the germs could be destroyed by pod disinfection and a method of control devised that would avoid the necessity of drying the beans after shelling. The results of various pod treatments are shown in table 3.

TABLE 3. *Pod disinfection of green lima beans*

Lot	Storage: Period	Storage: Temperature	Pod treatment before shelling and storing	Condition after storage: Spots per bean	Condition after storage: Stickiness	Condition after storage: Injury
	Days	° *F.*		*Number*	*Percent*	*Percent*
1	(1)	(1)	None	25	35	0
			1 percent HCl, 10 minutes	20	5	15
2	(2)	(2)	None	50	60	0
			10 percent $C_2H_4O_2$, 1 minute	7	0	15
			10 percent $C_2H_4O_2$, 1 minute	0	0	30
			0.5 percent $CuSO_4$, 5 minutes	2	0	0
3	7	50	None	52	58	0
			50 percent C_2H_5OH, 5 minutes, then washed	2	10	0
			1 percent CH_2O, 5 minutes, then washed	5	7	0
			1 percent CH_2O, 8 minutes, then washed	10	10	0
4	7	50	None	5	30	0
			30 percent C_2H_5OH, 2 minutes; in moist chamber 3 minutes	0	20	0
			1.6 percent CH_2O, 2 minutes; in moist chamber 3 minutes	0	0	0
			1.5 percent NH_4OH, 2 minutes; in moist chamber 3 minutes	0	30	0
5	6	50	None	5	30	0
			1.6 percent CH_2O, 2 minutes	0	0	0
			1.6 percent CH_2O, 2 minutes; in moist chamber 1 minute	0	0	7
			1.6 percent CH_2O, 2 minutes; in moist chamber 3 minutes	0	0	5
			1.6 percent CH_2O, 10 seconds; in moist chamber 3 minutes	0	0	0
6	(3)	(3)	None	25	45	0
			0.5 percent HCl, 5 minutes	20	15	0
			0.5 percent $CuSO_4$, 5 minutes	3	0	0
			0.5 percent $CuSO_4$, 5 minutes, then washed	15	30	0
			1 percent HCl, 5 minutes, then washed	10	5	0
			10 percent $C_2H_4O_2$, 5 minutes, then washed	10	7	0
			0.5 percent $CuSO_4$ + 2 percent $C_2H_4O_2$, 5 minutes, then washed	10	5	0
7	6	47–70	None	35	50	0
			1 percent HCl, 5 minutes	15	2	0
			1 percent $CuSO_4$, 5 minutes	8	0	5
			0.5 percent $CuSO_4$, 11 minutes	4	10	7
			1 percent HCl, 5 minutes, then washed	8	15	0
			1 percent $CuSO_4$, 5 minutes, then washed	8	20	0
8	7	45–50	None	25	60	0
			50 percent C_2H_5OH, 5 minutes, then washed	0	8	0
			2 percent $CuSO_4$, 5 minutes, then washed	20	20	2
			2 percent HCl, 5 minutes, then washed	10	5	0

[1] Lots 1 to 9, inclusive, and 15 were Fordhook beans from Florida or California; lots 10 and 12, Fordhook beans from Virginia; lot 11, Dreers Bush lima from Virginia; and lots 13 and 14, Fordhook type from Cuba.

[2] Stored for 5 days at 40° F., and then 1 day at 70°.

[3] Stored for 1 day at 70° F., and then 4 days at 50°.

TABLE 3.—*Pod disinfection of green lima beans*—Continued

Lot	Storage		Pod treatment before shelling and storing	Condition after storage		
	Period	Temperature		Spots per bean	Stickiness	Injury
	Days	*° F.*		*Number*	*Percent*	*Percent*
9	8	50	None	96	50	0
			1.6 percent CH_2O, 1 minute; in moist chamber 3 minutes	1	0	0
			1.6 percent CH_2O, 2 minutes; in moist chamber 5 minutes	0	0	0
			1.6 percent CH_2O, 1 minute; in moist chamber 3 minutes, then washed	17	5	0
			1.6 percent CH_2O, 5 seconds; in moist chamber 3 minutes, then washed	27	5	0
			1.6 percent CH_2O, 1 minute; then washed	25	30	0
10	8	50	None	47	35	0
			1 percent CH_2O, 1 minute	0	0	0
			4 percent CH_2O, 1 minute; in moist chamber 5 minutes	0	0	2
			1.6 percent CH_2O, 1 minute; in moist chamber 3 minutes	0	0	2
			1 percent HCl, 1 minute; in moist chamber 3 minutes	30	10	0
			1.6 percent CH_2O + 1 percent HCl, 1 minute; in moist chamber 3 minutes	0	0	1
11	6	50	None	80	50	0
			Washed in water	65	30	0
			4 percent CH_2O, 1 minute; in moist chamber 3 minutes	0	0	3
			1 percent CH_2O, 7 minutes; in moist chamber 5 minutes	0	0	27
			1.6 percent CH_2O, 1 minute; in moist chamber 3 minutes	0	0	2
			1.6 percent CH_2O + 1 percent HCl, 1 minute; in moist chamber 3 minutes	0	0	8
			4 percent CH_2O, 1 minute; in moist chamber 3 minutes, then washed	0	0	2
			1 percent CH_2O, 7 minutes; in moist chamber 5 minutes, then washed	0	0	7
			1.6 percent CH_2O, 1 minute; in moist chamber 3 minutes, then washed	0	0	2
			1.6 percent CH_2O + 1 percent HCl 1 minute; in moist chamber 3 minutes, then washed	0	0	4
12	6	50	None	8	70	0
			5 percent $C_2H_4O_2$, 3 minutes; in moist chamber 5 minutes	0	0	20
			2 percent $C_2H_4O_2$, 2 minutes; in moist chamber 5 minutes	0	0	8
			2 percent HCl, 2 minutes; in moist chamber 5 minutes	0	0	12
			1 percent HCl, 2 minutes; in moist chamber 5 minutes	0	0	4
			1 percent HCl + 0.5 percent CH_2O, 2 minutes; in moist chamber 5 minutes	0	0	4
13	8	50	None	40	60	0
			3 percent chlorinated lime 1 minute; in moist chamber 5 minutes	0	5	0
			3 percent chlorinated lime + 1 percent HCl, 1 minute; in moist chamber 5 minutes	0	5	0
14	6	50	None	7	55	0
			5 percent chlorinated lime 1 minute; in moist chamber 5 minutes	0	0	0
			3 percent chlorinated lime 1 minute; in moist chamber 5 minutes	0	5	0
			3 percent chlorinated lime + 1.25 percent HCl 1 minute; in moist chamber 5 minutes	0	3	1
			1 percent HCl 1 minute; in moist chamber 5 minutes	0	3	0
17	6	50	None	20	100	0
			5 percent chlorinated lime 1 minute; in moist chamber 5 minutes	0	5	2
			5 percent chlorinated lime 1 minute; in moist chamber 5 minutes, then washed	0	7	0
			1 percent chlorinated lime 1 minute; in moist chamber 5 minutes	0	5	0
			1 percent HCl 1 minute; in moist chamber 5 minutes	0	3	0

In a number of the tests the beans were held in a moist chamber for several minutes following the washing treatment. The purpose of this was to simulate the conditions that would occur with larger lots where the dipped pods might have to stand in baskets or in a pile for a short period before shelling.

Immersing the pods in a 4- or a 1.6-percent formaldehyde solution for 1 minute or 2 minutes usually gave good control of both stickiness and spotting with little or no injury to the beans, but it was found that the pod treatment was but little better than the bean treatment from the standpoint of objectionable residue. Formaldehyde to the amount of 1 part or more in 1,000,000 was found in the beans after shelling even when the disinfecting solution was reduced to 1 percent and the pods washed before shelling.

Immersing the pods in a 2-, 1-, or 0.5-percent solution of copper sulphate for 5 minutes gave good control in some instances but poor control in others. In some of the experiments there was slight injury, but no test was made as to the extent to which the copper sulphate passed over to the beans. In none of these treatments was there any evident effect upon color.

Immersing the pods in a 1.5-percent solution of ammonium hydroxide gave satisfactory control of spotting with no injury to the beans but failed to decrease stickiness.

Washing the pods in a solution containing 1 or 2 percent hydrochloric acid or 2 or 5 percent of acetic acid usually gave satisfactory control of stickiness and considerable reduction in spotting. With the stronger solutions and with prolonged exposure to the weaker solutions injury was sometimes found on the beans, but the results indicate that an acid wash has practical possibilities, especially where stickiness is the major problem, as is usually the case.

Pods were washed in a 30-percent solution of alcohol in one instance and in a 50-percent solution in another. The weaker solution gave considerable reduction of both diseases and the stronger still better control, but the results indicated that a more efficient fungicide was desirable in the case of pod treatments.

Chlorinated lime solutions were prepared according to the methods recommended for disinfection. Complete control of spotting and satisfactory commercial control of stickiness was obtained with 3-, 4-, and 5-percent solutions with no significant injury. The chlorinated lime solutions were prepared from stock material containing 53 percent of available chlorine.

All things considered, a chlorinated lime wash for the pods seems to meet commercial requirements better than any other disinfecting treatment that has been tested.

CAUSAL ORGANISMS

It is evident from the foregoing experiments that the stickiness developing on shelled lima beans is caused by bacteria and the spotting by a fungus. Studies have been made of the causal organisms with the purpose of determining their proper classification.

BACTERIA CAUSING STICKINESS

Isolations from sticky beans showed that there were a number of different bacterial organisms present on the surface. While most of these organisms caused an increase in stickiness when applied to the beans, it was decided to confine the detailed bacteriological studies to the three that were most active in producing the trouble, one each from California (Calif. 2), Virginia (Va. 9), and Florida (Fla. 2).

The writers made bacteriological and physiological studies of the three organisms, but it seemed advisable to have them studied and classified by some one familiar with bacterial soil organisms. The authors are indebted to N. R. Smith, of the Division of Soil Microbiology of the United States Department of Agriculture, for the identification of the organisms and for the supporting statements of the descriptions. Their own results are in agreement with the characteristics reported.

The bacterial cultures of Va. 9 have been found to have the following characteristics:

Small, slender rods, actively motile, with one to three polar flagella; Gram-negative.
Gelatin stab: Growth filiform, no liquefaction.
Beef agar: Abundant, flat, thin, glistening growth, the medium turning green fluorescent.
Broth: Cloudy, tender pellicle, flocculent sediment, green fluorescent.
Litmus milk: No coagulation, alkaline.
Milk agar plate: Large spreading colony, yellowish and brownish; no digestion.
Potato: Abundant, dirty-brown, glistening growth.
Indol not formed.
Nitrates not reduced.
Starch not hydrolyzed.
Acid from dextrose and glycerin; no fermentation of sucrose, lactose, dextrose, glycerin, nor mannitol.
Aerobic, slightly facultative.
No capsules; no spores.
Optimum temperature, 25° C.; minimum, 2°; maximum, 37°; thermal death point about 60°.
The size of the organism varied with cultural conditions. On beef agar 24-hour cultures the measurements were 0.6μ to 1.0μ by 1.0μ to 2.6μ (fig. 14, *A*).
Identified as *Pseudomonas ovalis* (Ravenel) Chester.
The organism has a soil habitat, but it has been found in the intestinal canal.

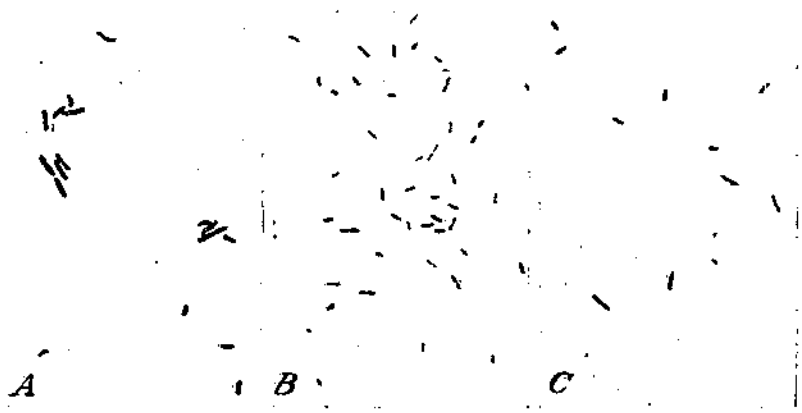

FIGURE 14.—Bacteria from 24-hour cultures on beef agar, × 1,400. *A*, Bacteria from Virginia beans, identified as *Pseudomonas ovalis*; *B*, bacteria from California beans, probably *Achromobacter coudunadum*; *C*, bacteria from Florida beans, resembling *A. lipolyticum* but probably not identical.

The bacterial cultures of Calif. 2 have been found to have the following characteristics:

Small, slender rods, actively motile, with one or two polar flagella; Gram-negative.
Gelatin stab: Crateriform, becoming stratiform.
Beef agar: Abundant, gray, glistening growth.
Broth: Cloudy, friable pellicle, slight flocculent sediment.
Litmus milk: Coagulated; whey extruded, slightly acid.

Milk agar plate: Compact, thick colony surrounded by a cleared zone half as wide as the colony.
Potato: Abundant, spreading, yellowish, glistening growth.
Nitrates not reduced.
Starch hydrolyzed.
No acid from dextrose, lactose, sucrose, starch, mannitol, nor glycerin.
Strong production of hydrogen sulphide.
Aerobic, facultative.
No capsules; no spores.
Optimum temperature, 25° to 30° C.; minimum, 2°; maximum, 37°; thermal death point about 56°.
On 24-hour beef agar cultures the rods measured 0.6μ to 0.9μ by 1.0μ to 2.4μ. (fig. 14, *B*).
Probable identity: *Achromobacter coadunatum* (Wright) Bergey et al.

The bacterial cultures of Fla. 2 have been found to have the following characteristics:

Small, short rods, motile, with peritrichous flagella; Gram-negative.
Gelatin stab: Crateriform, becoming stratiform.
Beef agar: Circular, smooth, translucent, spreading, very slimy.
Broth: Heavy clouding, friable pellicle, granular sediment, solution slimy.
Litmus milk: Coagulated, extruded whey, alkaline.
Milk agar plate: Growth spreading over plate, digesting milk ahead of it until whole plate is cleared.
Potato: Scant, dry, dirty-brown growth.
Nitrates reduced to nitrites.
Starch hydrolyzed.
Acid from dextrose, starch, mannitol, and glycerin, but not from lactose; no fermentation of sucrose, lactose, dextrose, glycerin, nor mannitol.
Aerobic, facultative.
No spores; no capsules.
Optimum temperature, 35° C.; minimum between 5° and 10°; maximum, 42°; thermal death point about 58°.
On 24-hour beef agar cultures the rods measured 0.6μ to 1.0μ by 0.8μ by 3.6μ.
Identification not accomplished. Resembles *Achromobacter lipolyticum* (Russ) Bergey et al. but probably not the same organism (fig. 14, *C*).

CLADOSPORIUM CAUSING SPOTTING

Isolations from the spots on different varieties of lima beans and from lima beans originating in Florida, Cuba, California, Georgia, and Virginia gave cultures of *Cladosporium* in all cases. In marked contrast with the organisms causing stickiness, all of the cultures appeared to be identical, or so closely related that it was impossible to place them in separate species. After holding the shelled beans for 4 to 6 days at 50° F. or above, *Rhizopus*, *Penicillium*, and *Fusarium* were occasionally found but without any relation to the spotting previously described.

On string bean dextrose and Thaxter's agar well established colonies of the *Cladosporium* have a white border and a dark greenish olive (*7*) center that has a velvety appearance, due to the abundant production of conidia. At first the hyphae (fig. 15, *A*) are hyaline, moderately branched and septate, but with age they become olivaceous and intricately branched, and the cells swollen and closely septate. The aerial hyphae are usually 3μ to 4μ in diameter, whereas those in the substratum may have a diameter as great as 7μ.

On lima beans and on most nutrient material only the Hormodendron type of spore production was observed (fig. 15, *B*, *C*, and *D*). The spore clusters are dense and correspond to those described by Robak (*8*) as arbor-shaped. The conidiophores are first hyaline, then golden olivaceous. They are septate, unbranched, erect or nearly so, and may arise singly from individual hyphae or in tufts from a mass of nodular-celled hyphae.

One or more basal conidia arise from the apex of the conidiophore by a process of budding (fig. 15). These basal conidia are usually larger than the others and some regard them as a part of the conidiophore, but they shatter as freely as the others of the conidial chain and germinate readily to form new growth. Other conidia develop from these basal conidia by a process of budding and these in turn develop other conidia. Two frequently arise simultaneously from the same plane, giving the spore cluster the general appearance of dichotomous growth (fig. 15, *D*). The most active budding occurs from the terminal conidia, but budding may also continue at various points on the conidial chains, adding to the complexity of the spore cluster.

At first the conidia are nearly hyaline but later become dark golden. On string bean dextrose agar the basal conidia and the first two or three that arise from them are cylindrical. Proceeding out the chain the spore forms shift to oblong-ellipsoid, spindle, ellipsoid, and subround. The basal conidia are 12μ to 22μ by 4μ to 5μ and often once or twice septate. The conidia other than the basal are 5μ to 8μ by 4μ to 5μ and rarely once septate.

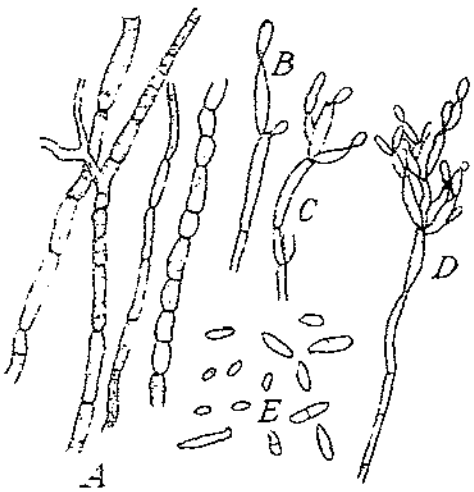

FIGURE 15.—*Cladosporium* on string bean dextrose agar. *A*, Various forms of mycelium; *B*, *C*, and *D*, early stages of conidial production. × 450.

On water agar, under otherwise favorable conditions, the spore sizes are similar to the above but with a slight lowering of the minimum measurements.

When water agar cultures are held at a temperature above the optimum or when the medium undergoes considerable drying after the establishment of the fungus, a different type of spore production occurs. The conidiophores are septate, of a golden color, and either simple or branched. The conidia are golden in color, often once septate, and measure 7μ to 12μ by 5μ to 8μ (fig. 16).

The above descriptions are in sufficient agreement with those reported by Bennett (*1*), Robak (*8*), and others to justify the classification of the fungus as *Cladosporium herbarum* (Pers.) Link.

The isolation of the fungus from beans grown in such widely separated points as California, Florida, Cuba, Georgia, and Virginia is in agreement with the fact that the fungus *Cladosporium* is known to have a wide and general distribution.

No evidence of parasitism on beans in the field has been found, but the fungus was found in abundance on dead and dying bean pods at the Arlington Experiment Farm, Arlington, Va.

DISCUSSION

The above experiments seem to leave no doubt as to the nature and cause of stickiness and spotting of fresh green lima beans. They show that stickiness is due to bacteria and the spotting to a fungus. The

causal organisms are types occurring in the soil or on decaying vegetable matter. They are carried on the pods and are spread to the beans in the process of shelling. Proof of this is found in the fact that stickiness and spotting are not problems with unshelled beans, and that disinfecting treatments of the pods before shelling prevent the development of the troubles on the beans after shelling.

Four different methods of control have been found: Holding the shelled beans at low temperature; exposing them to carbon dioxide as a supplement to refrigeration; disinfecting the beans; and disinfecting the pods before shelling.

Of these methods low temperature has the most points in its favor, at least from the standpoint of maintaining a high-grade product. Shelled green lima beans sustain a rapid loss in sweetness, freshness, and quality when held at high temperatures, as recently shown by Carolus (4). It is important, therefore, apart from control of stickiness and spotting, that the beans be held at as low a temperature as practicable. The present experiments indicate that a temperature of 40° to 41° F. should give 6 to 7 days for shipping and marketing without the development of spotting and with little or no development of stickiness. A temperature of 32° doubles this period of safety.

FIGURE 16.—Abnormal growth of *Cladosporium* due to drying of medium, water agar. *A*, × 450; *B*, × 240.

Where it is impossible to secure prompt and satisfactory refrigeration, the supplementary use of solid carbon dioxide offers a practical means of control. The presence in the storage atmosphere of 25 percent or more of carbon dioxide delayed the development of stickiness as much and the development of spotting more than a drop in temperature of 18° F. Such accumulations of gas can be secured more readily and much more quickly than the equivalent drop in temperature, especially in the case of cooling from temperatures of 50° or 60° to temperatures 18° lower. Temperatures of 40° and lower are difficult to secure under commercial shipping conditions. The carbon dioxide treatments have been found to have a favorable effect upon quality similar to that obtained by refrigeration, and shipping tests have shown that initial carbon dioxide treatments can be readily adapted to either pony-refrigerator or refrigerator-car conditions.

Exposure to low temperature or to carbon dioxide delays the development of the bacteria and fungi but does not destroy them. Washing the beans in a solution of ethyl alcohol and washing the pods in a solution of chlorinated lime have been found to be satisfactory disinfecting treatments for the removal of the causal organisms and may well have a commercial value even when refrigeration is to be employed later.

SUMMARY

The spotting that develops on shelled green lima beans has been found to be caused by a fungus and the stickiness by bacteria, both of which are spread to the beans in the process of shelling.

Lowering the humidity of the storage atmosphere caused a decrease in both stickiness and spotting but did not give satisfactory control.

Temperature studies were made on stickiness and spotting and on the causal organisms. A temperature of 50° F. did not always hold the troubles in check for a period of 4 days, but a temperature of 41° held them in check for 6 to 7 days or longer, and a temperature of 32° for 10 to 14 days.

Holding the beans in an atmosphere containing 25 percent or more of carbon dioxide had an inhibiting effect upon the development of stickiness equivalent to that of an 18° F. drop in temperature and a still greater effect in the control of spotting with no unfavorable effect upon flavor.

Washing the beans in a 30-percent solution of ethyl alcohol or washing the pods in a 4-percent solution of chlorinated lime gave complete control of spotting and good commercial control of stickiness.

A considerable variety of bacterial organisms were isolated from the beans and found capable of producing stickiness. Three of the most active of these were *Pseudomonas ocalis*, from Virginia beans; an organism with the probable identity of *Achromobacter coadunatum* from California beans; and an organism resembling *Achromobacter lipolyticum* from Florida beans.

The fungus causing spotting was identified as *Cladosporium herbarum*.

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