T H E S I S

FLOURESCENCE IN DRY BEAN SEED

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY H. H. FISHER

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Chapter I

INTRODUCTION

Like many other crops, beans sustain great losses annually from diseases. In 1938 the loss in the United States ranged from a trace in Texas to 69 percent in Nebraska with an average for the entire country of 12 percent. Upon reviewing literature pertaining to the diseases of beans, this author found that the seed-borne diseases are among those which cause the greatest losses. However, the bacterial diseases and virus diseases are the most important. The seed-borne diseases are listed as follows: Fungus Diseases - Anthracnose, Ashy Stem Blight, and Watery Soft Rot; Bacterial Diseases - Common Blight, Halo Blight, and Wilt; and Virus Diseases - Common Bean Mosaic and Bean Mosaic Virus 4A.

Since the presence of a seed-borne disease, haircracks and other injuries on dry bean seed are not always detectable with the naked eye, the growers and dealers are in need of a method of testing the seed for such conditions. Inasmuch

as ultra-violet light has been used in the identification of both bacterial and virus infections in other plants, it was the purpose of this work to determine if this light could also be made of use in the detection of seed-borne diseases of beans.

Chapter II

REVIEW OF LITERATURE

The literature on the fluorescence of substances under ultra-violet light is not voluminous and much of it is printed in foreign languages and obscure publications. Literature on the fluorescence of plant materials is especially scarce.

The work of Radley and Grant (18) is largely a compilation of the works of others and arranged according to subject. Under the subject of seeds they describe the difference in fluorescence of several strains of barley when examined under ultra-violet light. By their differences in fluorescence the grains could be divided into groups, according to chemical and biological characteristics.

Also, the difference in fluorescence between fresh, sound seeds and old or damaged seeds was described. Many seeds showed no differences between new and old seeds.

This reference suggested to the author of this paper the possible use of ultra-violet light to detect strain and age differences among Pinto bean seeds.

Gentner (4) used a mercury arc in quartz with a filter which transmitted 3,000 to 4,000 Ångstroms to test the applicability of qualitative fluorescence analysis in practical seed testing. Only a few of the many hundreds of species of seeds tested showed fluorescence. The fluorescence of some of the species was described but Phaseolus vulgaris did not appear in this description. The majority of the species fluoresced when crushed and the types of fluorescence of crushed peas, vetches, and lentils were noted.

In general, seeds which had been attacked by fungi and bacteria during germination frequently fluoresced in brilliant colors but this fluorescence disappeared when the seeds were dried.

This article was inconclusive but it gave some suggestions as to what might be expected when seeds were examined under ultra-violet light.

In a publication by Haitinger (5) 1928, mention was made of the greenish blue fluorescence of bean meal and the rosy fluorescence of pea meal. He reports that when a piece of filter paper was soaked in an alcoholic NaOH extract of the bean meal the paper fluoresced white with a bluish "shimmer" and the margin of the paper appeared blue. When a piece of filter paper was soaked in a similar extract from pea meal, it fluoresced yellow with a weak rose "shimmer" and the margin of the paper fluoresced blue.

A description of the rosy fluorescence of pea meal and the bluish-green fluorescence of bean meal was published by Neseni (15) 1932, in one of his earlier publications. In a later work, Neseni (16) 1934, described in detail the fluorescence of dried peas. He stated that the majority of the dry peas fluoresced a rose color. Extracts with alcohol, chloroform, or acetic acid did not show the fluorescence of the extracted peas. Solutions of

the pigment layered with concentrated H_2SO_4 fluoresced in a violet-red ring, which upon shaking became a deep, wine red. Some of the peas showed external points or flakes which fluoresced a strong sulphur yellow color as well as black. When such peas were cultivated on beer-wort agar, it was shown that the yellow fluorescence was partly due to bacteria and partly to fungi. However, he did not identify those organisms. He concluded that the ultra-violet lamp was helpful in establishing the presence of fungal infections.

Leskov (10) and Levaditi (11) in some detail described the fluorescent characteristics of several bacteria and fungi. This, together with the previously cited work of Neseni (16) indicated the possibility of the fluorescence of dry beans being used to detect the presence of micro-organisms.

The use of ultra-violet light for the detection of diseases in potatoes was suggested by Flint and Edgerton (3) in their work with ring rot of potatoes. Hervey (8) published similar results on the same subject at about the same time. At a later date Iverson and Harrington (9) reported on

the accuracy of the ultra-violet method for selecting ring rot free potato seed stocks and McLean and Kreutzer (13) described a fluorescence peculiar to virus infected potato tubers.

It was the above cited literature that suggested to this author the possibility of using ultra-violet light for the detection of virus infections in dry beans.

For the supplemental experiments in this work, the attempted extraction of the fluorescent material from the bean seeds was suggested by the work of Best (1). In that publication Best stated that he was not only able to isolate the fluorescent material from tobacco, but he was able to identify it as 6-methy-7-hydroxy 1:2 benzopyrone.

In the anatomical studies of the bean seed, the descriptions and plates by Lute (12) and Pammel (17) were found invaluable for identifying the tissues of seeds.

The method of Boswell, Toole, Toole, and Fisher (2) was used in an attempt to trace any change in composition of seed during storage which might give some suggestion as to the cause of the fluorescense of bean seed.

Chapter III

METHODS AND MATERIALS

A Portable Black Light, Model 70, produced by Switzer Brothers and equipped with a GE 250 W, A-H 5, mercury vapor lamp, was used in this work. This lamp with its filter generated ultra-violet radiation in the region between 3,300 and 4,000 Angstroms--the principal line being 3650 Å. For "black light" application, the absence of visible light is essential, and this visible light was absorbed by the use of a red-purple filter.

One pound samples (approximately 1,600 seeds per pound) of each of 12 varieties of dry beans were examined under ultra violet light in a darkened room. The fluorescence of these varieties is described in Table 1. A small number of the Pinto beans contained small spots of various sizes which fluoresced a lemon yellow. Because of that distinctive characteristic, this variety was selected for further study.

To determine whether the lemon yellow fluorescence of Pinto beans was related to the

TABLE 1.--VARIETIES OF DRY BEANS UNDER TUNGSTEN AND ULTRA-VIOLET LIGHT COMPARED WITH COLOR STANDARDS OF RIDGEWAY (19)

Variety	Tungsten Light	Ultra-Violet Light
Bountiful	Tan	Brown
Brittle Wax	White	White, lighter areas
Giant Stringless	Brown	Brown
Great Northern	White	White, lighter areas
Henderson Bush	White	White, lighter areas
Improved Kidney Wax	White	White, lighter areas
Longreen	Blue mottle	Deep blue
Pinto	Brown mottle	Pale violet, brown, yellow
Plentiful	Black	Black
Refugee 1066	Blue mottle	Deep blue
Stringless Black Valentine	Black	Black
Top Notch	White	White, lighter areas

presence of pathogenic bacteria, samples of bean seeds containing bacterial wilt, halo blight, and common blight were examined under ultraviolet light. To prove the presence of these diseases, these dry seeds known to be diseased were surface sterilized by rinsing in a solution of one-tenth percent mercuric chloride, followed by several rinses of sterile water. Following this, each bean was placed in an individual test tube of sterile water and allowed to soak for 24 hours. The beans were then broken open with a scalpel to allow the bacteria to escape into the water and a 3 mm loop of the water from each tube was streaked across plates of sterile agar.

To test the pathogenicity of the cultures obtained from the above described experiment, Pinto bean plants which had just produced the first set of trifoliate leaves were selected for inoculation. A drop of the culture was placed on the cotyledonary node and the stem was pierced through the drop with a flat-headed needle.

One hundred dry seeds each of fluorescent and nonfluorescent Pinto beans were treated in identically the same manner as those known to contain the bacterial diseases named above. The procedure was repeated five times.

The above described pathogenicity tests were conducted with the cultures isolated from the fluorescent and nonfluorescent beans.

One hundred Pinto beans harvested from mosaic-infected plants were examined for fluorescence. These, together with 500 each of the fluorescent and nonfluorescent seeds, were grown in soil in the greenhouse for 25 days and observed for virus infection.

Analysis of variance using the F values from Snedecor (21) was applied to the data in

Tables 2, 4, and 5.

Chapter IV

RESULTS AND DISCUSSION

None of the beans which contained bacterial wilt, halo blight, or common blight fluoresced when examined under ultraviolet light--neither did they appear differently from the healthy seeds. Confirmation that each of the samples actually did contain the organism of which it was suspected was obtained when cultures of the pathogens were isolated from the seeds and grown on agar plates. The pathogenicity of each culture was proven by inoculating healthy plants with the culture. Each inoculated plant produced symptoms typical of the disease caused by the respective pathogen.

When the same procedure which was used in isolating bacteria from the infected seeds was applied to the fluorescent and nonfluorescent beans, two types of bacterial cultures were obtained. One type was yellow in color and the other white. It is shown in Table 2 that there was no significant

TABLE 2.--TYPES OF BACTERIAL CULTURES OBTAINED FROM NONFLUORESCENT AND FLUORESCENT BEANS. 100 SEEDS USED IN EACH TRIAL.*

NONFLUORESCENT

Trial	White Cultures	Yellow Cultures	Total
1	2	1	3
2	0	2	2
3	10	6	16
4	3	2	5
5	4	2	6
Total	19	13	32
Mean	3.8	2.6	3.2

FLUORESCENT

Trial	White Cultures	Yellow Cultures	Total
1	3	2	5
2	4	1	5
3	2	6	8
4	1	0	1
5	3	2	5
Total	13	11	24
Mean	2.6	2.2	2.4

*NO SIGNIFICANT DIFFERENCE BETWEEN TYPES OF CULTURES OR TYPES OF SEED.

difference in the numbers of cultures of either organism isolated from either the fluorescent or the nonfluorescent beans.

Using wilt and common blight pathogens as checks, tests for pathogenicity were applied to both the yellow and the white bacterial cultures. As shown in Table 3, neither the yellow nor the white cultures were pathogenic to beans. Upon discussing these results with the other workers in bacterial diseases of beans, it was learned that both of the nonpathogenic white and yellow cultures are commonly obtained when attempting bacterial isolations from beans. The reason for their presence or their identity was not learned--merely the fact that they are commonly found in beans and are nonpathogenic.

The 100-bean sample harvested from virus infected plants showed about the same percentage of fluorescence (1.5 per cent) as did all the healthy Pinto beans when examined under ultraviolet light.

At the end of the 25 day growing period, 11 per cent of the plants grown from the seeds

TABLE 3.--PATHOGENICITY TESTS OF YELLOW AND WHITE BACTERIAL CULTURES ISOLATED FROM FLUORESCENT AND NONFLUORESCENT BEANS. CORYNEBACTERIUM FLACCUMFACIENS AND PSEUDOMONAS MEDICAGINIS VAR. PHRASEOLICOLA CULTURES OF KNOWN PATHOGENICITY USED TO TEST METHODS.

Culture	Plants Inocultated	Plants Infected	% Pathogenicity
White	10	0	0
Yellow	10	0	0
Ps. medicaginis var. phaseolicola	10	8	80
Corynebacterium flaccumfaciens	10	10	100

of virus infected plants had produced virus symptoms. According to Harter and Zaumeyer (6) these results were not abnormal since as high as 50 per cent infection is rarely observed in plants grown from seed harvested from virus infected parents.

Of the plants grown from the 500 fluorescent and the 500 nonfluorescent beans, none showed virus symptoms. It is possible that virus infections were present but masked. However, the author believed this unlikely since the other plants from known virus infected seed expressed symptoms under the same growing conditions.

From this experiment the author concluded that there was no association between the fluorescence of dry Pinto beans and infections of bacterial wilt, halo blight, or common blight; and that it was unlikely that the fluorescence was associated with a virus infection.

SUPPLEMENTAL EXPERIMENTS

1. The seed coats were peeled from fluorescent beans and the seed coats and cotyledons were ground separately in a burr mill. The ground seed coats still fluoresced a lemon yellow. The same procedure was followed using nonfluorescent seeds. All of the ground samples were placed separately in a Bailey-Walker extractor to which ethyl ether was added.

Continuous extraction took place for 24 hours. At the end of the extraction period the samples and their extracts were examined under ultra-violet light. All of the samples fluoresced the same as they did prior to extraction. The extracts of all the samples fluoresced a pale blue.

One hundred grams of whole fluorescent beans were placed in a Soxhlet extractor to which acetone was added. After eight hours of extraction the beans fluoresced the same as they did before extraction and the extract fluoresced a pale blue. The procedure was repeated using nonfluorescent beans and the extract fluoresced in the same manner as did that from the fluorescent beans.

The same experiment was repeated using fresh samples of fluorescent and nonfluorescent beans and ethyl alcohol. The results were the same.

Ten fluorescent beans were placed in a petri dish and covered with water. After 16

hours, eight of the beans had begun to swell but two had not. The fluorescence of the swollen beans was still visible but much fainter. The two unswollen beans retained their original fluorescence. After 24 hours of soaking, the fluorescence had completely disappeared from the eight swollen beans but the two unswollen beans still retained their original fluorescence. The water fluoresced pale blue similar to that of the either, acetone and alcohol extracts. The water extract from a sample of nonfluorescent beans fluoresced the same pale blue as that from the fluorescent beans.

CONCLUSION: The yellow fluorescent material in dry Pinto beans is not soluble in ethyl ether, acetone, or ethyl alcohol. It is probably water soluble or in some way changed since the yellow fluorescence was removed after the beans were soaked 24 hours.

2. To determine whether or not the yellow fluorescence of Pinto bean seeds was due to a strain

difference, 13 strains of Pinto beans were selected for study. A 500 seed sample was taken from each strain. Each sample was divided into 100 seed replications. Each replication was examined under ultra-violet light and the number of fluorescent beans was recorded. The results were compiled in Table 4. No significant differences as to the number of fluorescent beans within each strain were found when analysis of variance was applied to the data.

3. Germination trials were conducted to determine if any significant difference existed in germination between fluorescent and non-fluorescent Pinto beans. Five hundred seeds each of fluorescent and nonfluorescent beans were divided into replications of 100 seeds each and planted in soil in the greenhours. This procedure was repeated five times. Normal, baldheads, and weak plants were counted separately. The results are shown in Table 5. When the numbers of normal seedlings, bald-

TABLE 4.--NUMBERS OF FLUORESCENT BEANS IN 500 EACH OF 13 STRAINS OF THE PINTO VARIETY. EACH LOT WAS DIVIDED INTO REPLICATIONS OF 100 SEEDS EACH. *

STRAIN	REPLICATIONS					TOTAL	MEAN
No. 1	2	3	0	0	2	7	1.4
No. 4	0	0	2	3	1	6	1.2
No. 5	3	2	0	1	3	9	1.8
No. 6	0	0	3	3	1	7	1.4
No. 14	2	2	3	1	3	11	2.2
No. 15	0	2	2	2	0	6	1.2
No. 16	2	1	0	2	2	7	1.4
No. 20	2	1	2	0	0	5	1.0
Idaho	1	1	2	2	2	8	1.6
Idaho 78	3	2	1	0	0	6	1.2
Idaho 106	1	0	3	2	3	9	1.8
Idaho 118	3	2	1	0	1	7	1.4
Wyoming	0	0	3	2	2	7	1.4
TOTAL	19	16	22	18	20	95	

* NO SIGNIFICANT DIFFERENCES.

TABLE 5.--PERCENTAGES OF NORMAL, BALDHEADS, AND WEAK PLANTS FOUND IN FLUORESCENT AND NONFLUORESCENT PINTO BEANS. EACH TRIAL CONSISTED OF FIVE REPLICATIONS OF 100 SEEDS EACH. *

NONFLUORESCENT

Trial	Normal	Baldheads	Weak Plants
1	70.4	2.8	9.6
2	80.0	3.6	4.4
3	74.2	1.4	8.4
4	91.2	1.0	3.2
5	71.8	2.0	2.6
MEAN	77.5	2.2	5.6

FLUORESCENT

Trial	Normal	Baldheads	Weak Plants
1	65.2	4.8	10.0
2	79.8	2.6	5.0
3	80.8	3.4	7.8
4	82.6	2.6	4.0
5	82.8	3.0	3.2
MEAN	78.2	3.3	6.0

* NO SIGNIFICANT DIFFERENCES.

heads and weak plants were compared by analysis of variance, no significant difference was found between fluorescent and nonfluorescent beans.

4. Free hand sections were made of dry, untreated beans of each of the 12 varieties listed in Table 1. When these sections were examined under ultra-violet light, the cotyledons of all 12 varieties examined fluoresced the same bluish white. The only difference in fluorescence among these varieties was in the seed coats. To determine the location of the yellow fluorescence in the Pinto bean seed coat, fluorescent seed coats were peeled from dry Pinto beans, embedded in cellodidin and sectioned with a sliding microtome. The sections were mounted on slides in balsam. The same procedure was followed using nonfluorescent seed coats.

The ultra-violet light was placed directly beneath the stage of the microscope. With the substage condenser removed, the yellow fluorescent areas were observed to lay in the inner edge of the palisade layer, the subepidermol layer and the outer edge of the parenchyma. (See Figure 1). With the substage condenser added, the fluorescence was still visible but not as clearly defined. The light intensity was insufficient for photomicrography.

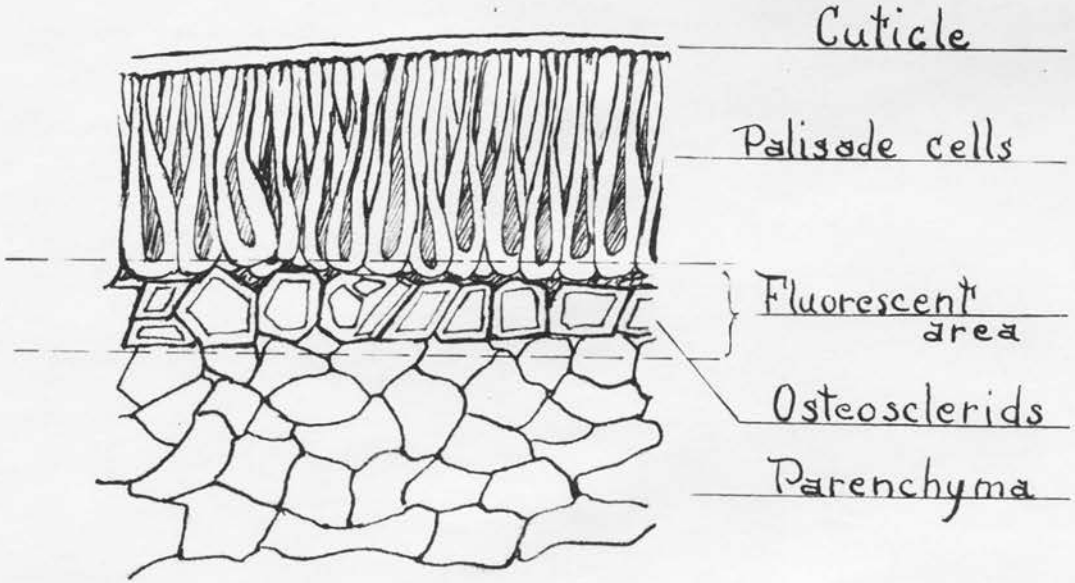

FIG. 1.--CROSS SECTION OF PINTO BEAN SEED COAT SHOWING LOCATION OF FLUORESCENT AREA. (CAMERA LUCIDA DRAWING)

Chapter V

SUMMARY

An attempt was made to use the ultra-violet light for the detection of seed-borne diseases of beans.

Dry seed from twelve varieties was examined under ultra-violet light. Because a small percentage of Pinto beans fluoresced a lemon yellow, that variety was selected for further study.

Both fluorescent and nonfluorescent beans were compared under ultra-violet light with beans believed, and later proven, to contain pathogens which cause bacterial wilt, halo blight, and common blight. None of the infected seed fluoresced.

Attempts to isolate organisms from the fluorescent and nonfluorescent beans were made. Only two types of nonpathogenic bacterial cultures were obtained.

Pinto beans harvested from virus infected plants were compared under ultra-violet light with the beans which were being used in the

experiment. The beans of both lots fluoresced in approximately the same proportion. These beans were grown in soil in the greenhouse for 25 days and examined for virus infections. Eleven percent of the plants grown from virus infected seed showed virus symptoms. No virus symptoms were observed among the other lots.

The author concluded on the basis of these preliminary exploratory investigations, that there was no association between the fluorescence of dry Pinto beans and infections of bacterial wilt, halo blight, or common blight, and that it is unlikely that the fluorescence is associated with a virus infection.

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