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CHOCOLATE SPOT ON *Vicia faba* CAUSED BY *Botrytis fabae*

SARDINA AND *B. cinerea* Pers.

A LITERATURE REVIEW

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1977

ENRIQUEZ, G. A.\* Chocolate spot on *Vicia faba* caused by *Botrytis fabae* Sardiña and *B. cinerea* Pers.; a literature review.\*\* Turrialba, Costa Rica, CATIE, 1977. 34 p. 51 ref.

#### RESUMEN

En la presente revisión se colecta datos históricos del cultivo de haba, se recalca su importancia en los diferentes países, su uso dentro de sistemas agrícolas en algunas zonas de América del Sur y Guatemala.

Se analizan los diferentes cultivos o plantas afectadas por el organismo patógeno (*Botrytis fabae* S.), su historia, sus sinónimos, su distribución geográfica e importancia económica.

Se revisa la sintomatología general de la enfermedad en todos los órganos de la planta donde afecta y varios aspectos fisiológicos y patológicos de las relaciones huésped-patógeno.

Se comenta extensamente sobre la Etiología, Epidemiología, Control del Organismo y finalmente se da una lista general de las enfermedades que se puede encontrar sobre el haba.

#### SUMMARY

The effect of *Botrytis fabae* S. on broad beans (*Vicia faba*) and other crops, its history, distribution and economic importance are presented.

This review present worldwide historical data on broad beans and comments on its importance in Latin America in intercropping systems with other food crops.

The syntomatology and the physiological and pathological host-pathogen relations of the disease are discussed. Etiology, Epidemiology and the control of the pathogen organism is also discussed. Other diseases that attack broad beans are listed.

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\*\* Document prepared at Cornell University, 1975.

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CHOCOLATE SPOT ON *Vicia faba* CAUSED BY *Botrytis fabae*

SARDIÑA AND B. cinerea Pers. A LITERATURE REVIEW \*

Gustavo A. Enríquez\*\*

INTRODUCTION

The broad bean (*Vicia faba*) is one of the most ancient of the cultivated esculents, having been grown by the ancients Greeks, Romans, Hebrews and Egyptians. It is said to have been introduced into China about 2822 B.C. (44).

Broad bean is an important plant in the cropping systems in Latin America. It is commonly grown with corn, potatoes and has been successfully intercropped with small grain cereals and chenopodiums.

In Ecuador most of the land cultivated above 3,000 m sea level have broad bean as the principal crops and in intercropping systems with potatoes, other tubers and chenopodiums (*Ch. quinoa* Willd.).

In Guatemala, beans and broad beans are planted simultaneously with corn and when they hill-up they plant a cucurbit. Most of the cropping is done between 2,000 and 2,500 m of altitude (M. Castillo, personal communication 1974).

*Vicia faba* has 12 and 14 chromosomes. It is also known as the horse bean, field bean, tick bean, Windsor bean, garden beans, great field beans (28, 37). The synonyms are: *Faba vulgaris* Moench., *Faba sativa* Bernh., *Vicia faba* L. for megalosperma Beck. *Vicia faba* L. subsp. mayor Alef. (28, 37).

*Vicia faba* is one of the oldest of cultivated plants. It originated in the Mediterranean region or South-Western Asia. Cultivars with seeds 1 cm long were widely cultivated in pre-historic times, and have been found in the remains of lake-dwellings in England and Switzerland. It was the only edible bean known in Europe in pre-Columbian times and was

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\* Document prepared at Cornell University, 1975

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introduced into the New World after 1492 (37). It is known that in 1602 was cultivated for the first time in the Atlantic Coast of U.S.A. (28). This bean is seldom grown in the United States since the summers are too hot in the South and winters in the North are so cold that it cannot be planted in the autumn and carried over. In some section of the South and of the Pacific Coast, the seed may be planted in the autumn or the winter for a spring crop, but other types of beans are so easily grown that the extra effort necessary to produce the broad bean does not seem to be justified. It can be grown towards the edge of tropics as a winter crop, as is done in the Northern Sudan and Burma or at high altitudes as in Latin America. Burkart said "the most beautiful plantations of broad beans I saw was in the Andean Valley of Jujuy (Caspalá, 3200 m over the sea level) where the plants were 2 m tall and were in production without any sign of parasites" (28).

In Southern Europe, the seed is often planted in the fall and the young plants are protected during the winter. It is grown in Abisinia to 3000 m of altitude. It is grown to some extent in California where it is planted in February and March in the warmer section and later in the cooler regions near San Francisco. It is grown mostly for local use and it is not of much importance (44).

Broad Beans are grown as garden cultivars for the green-shell beans and as a field crop for dried beans which are used as food for man and livestock. They are also grown for fodder and hay.

The broad bean is an important food crop in Latin America. The estimated hectareage devoted to the broad bean in 1970 was over 323.000 ha and the estimated hectareage for the World was 4.706.000 ha with a production of 5.181.000 metric tons of dry broad beans (42).

It is grown mainly at higher altitudes where the climate is relatively cool, it is not suited to the low hot tropics where it may flower well but usually produces no pods. It requires good fertile soils with a plentiful supply of lime and adequate and sustained water supply. It does well on heavy clay soils.

Broad bean is an erect, robust, leafy, glabrous, annual herb. 30-180 cm tall. Well developed tap-root to 1 m or more with strong laterals; smaller roots with large clusters of small lobed nodules. Stems stout, hollow, square, winged at angles, with 1-7 branches from axils of cotyledons and basal nodes, otherwise unbranched. Leaves pinnate, often ending in small points (rudimentary tendril); stipules half-sagitate, dentate, often with an extra floral nectary; leaflets 2 to 6, subopposite or alternate, ovate to elliptic somewhat glaucous, mucronate, 5-10 cm long. Inflorescences short axillary racemes 1-6 flowered. Flowers fragrant 2.5 to 3.7 cm long; calyx campanulate, oblique, teeth triangular; standard white, often faintly streaked with black on dorsal side, suberect, spatulate, longer than wings; wings often with purplish blotch; keel oblique, adnate to wings; stamens short, diadelphous; anthers uniform, usually dark coloured; style short, bent, with tuft of hairs near tip; stigma terminal. Pods stout, sub-cylindrical or flattened, beaked, 5-10 cm long in field cultivars, up to 30 cm long in garden cultivars, fleshy with white velvety lining when young, tough and hard at maturity. Seeds very variable in shape and size, strongly compressed to nearly globular, white, green, buff, brown, purple or black, 1-2.6 cm long; hilum prominent. Weight of 100 seeds 40-180 g. (37, 38).

The anthers dehiscence early and pollen is shaded in the keel, so that self-pollination can occur. Nevertheless, the flowers are much

visited by bees and cross-pollination can take place, as is shown by the considerable mixture of field beans in Europe (37).

There is a heteroblastic change in leaflet number in many stocks, the rate of change being affected by the temperature and photoperiod under which the plants are grown. In all except the earliest flowering stocks of broad beans, and particularly at high temperatures, flowers initiation shows a quantitative long day response. For full development of the initiated inflorescences, long days are required (33).

#### SUSCEPTS OF BOTRYTIS

B. cinerea, not infrequently occur in samples of red clover seed. The non-aggressive attack of the organism is also seen in the form of spots on the other host as on the petals of roses in the open, cyclamens under glass, on ripening tomatoes fruit, lettuce, in wet seasons Botrytis may damage potato haulm considerably as pointed out by Pethybridge in 1916, and may occasionally rot tubers in storage. Dry Eye Rot of young apples fruits has been shown to be due B. cinerea. The commonest cause of die-back of rose shoots is Botrytis, intervening after initial damage by frost or after pruning, some varieties are much more susceptible than others. The vines of grapes are attacked and the die-back in goosberry bushes (3).

B. cinerea attack lettuce, escarole, endive and globe artichoke, on the first three crops it affects young seedlings and contributes to damping-off, particularly in cold-frame and greenhouses culture, on all of them it is an importante transit disease. Several rots uncountered infrequently or of minor importance on muskmelons name gray mold rot. Gray mold of peas is found sometimes on pod peas in transit with a water-



soaked grayish-green spots. In potatoes a blight of foliage is sometimes incited by B. cinerea under cool, humid conditions, rot of tubers has been reported in storage and on shipments. Gray mold is the most destructive disease of rhubarb in transit and market, and is rare on sweet potatoes producing a soft semiwater rot with a starchy odor. Gray mold is a common transit and storage disease of tomatoes (47).

Several species of plants are known to be affected by B. cinerea Pers. (8, 18, 37, 50). Botrytis fabae appears to be specific for Vicia faba L. (39, 50).

### THE DISEASE

#### NAMES

The most common name is "Chocolate spot". In 1931 Rodríguez Sardiñas described two diseases of Vicia faba in Spain. One of these he attributed to Botrytis fabae Sar., the other to a form of B. cinerea Pers., it would appear probable that at least one of them is identical with Chocolate spot. In 1933 Ikata described a new species of Botrytis as the causal organism of red spot or chocolate spot disease of Vicia faba in Japan (49, 50). Mateo Box 1961 (28) mentioned the "Mal del esclerocio" caused by B. vulgaris.

#### HISTORY

The first reference to this disease in literature (50) is by Berkeley, 1849 who described an epidemic outbreak near Northampton in 1849. Further records were made by the same author in 1849, 1851, and 1975. Points of special interest raised were that no pathogen was found

associated with the lesions, the attacks were confined mainly to winter-sown broad beans and the disease was more prevalent after prolonged periods of wet weather and where crops were densely sown.

No detailed study of chocolate spot is found until Paine and Lacey, 1923 described Bacillus lathyri Manns and Taubeuh, as the causal agent of the disease. It is interesting to note the following points from their paper: B. lathyri was isolated from only about 20 percent of the lesions, many of the lesions contained no living organisms, while from others only common soils saprophytes were isolated. These authors state that infection by Botrytis sp. frequently followed attacks of chocolate spot. Riker and Riker 1932 isolated from chocolate spot lesions an organism which resembled Bacterium seminum (Cayley) Stevenson, the organism was vigorously pathogenic to broad beans and peas grown in the greenhouse. The authors tentatively suggested a causal connection between this organism and chocolate spot.

In 1931 Rodriguez Sardiña (39), described two diseases of Vicia faba in Spain. One of these was attributed to B. fabae Sard., the other to a form of B. cinerea Pers. The pathogenicity of these fungi was not limited to Vicia faba among the leguminosas. While the published descriptions and more particularly the photographs of these two diseases indicate that they differ slightly from chocolate spot as it occurs in other places (Britain), it would appear probable that at least one of them is identical with chocolate spot.

Nattrass in 1935, cited by Wilson (50), isolated a form of Botrytis closely resembling B. fabae Sard. from beans affected by chocolate spot in Cyprus. This lends support to the idea that at least one of the

diseases described by Rodriguez Sardiña was identical with chocolate spot. The papers published by Rodriguez Sardiña in 1929 and 1931 on the subject were confirmed largely to cultural studies of the causal organism. Artificial inoculation is described and brief mention is made that high humidities are essential for infection. In 1933 Ikata described a new species of Botrytis as the causal organism of red spot or chocolate spot disease of V. faba in Japan. The organism, which he named Botrytis fabae Ikata, is virulently pathogenic to V. faba but is apparently restricted to that host among the Leguminosae. The paper by Ikata is to a great extent, concerned with morphological and cultural studies of the pathogen and with extensive tests of fungicides in the control of the disease. Ikata mentions the occurrence of chocolate spot disease in Britain and concludes that the disease investigated by him is "included" in it. The diseases described by Rodriguez Sardiña and Ikata are attributed to specific forms of Botrytis, while Wilson (50) has found that the symptoms of chocolate spot may be induced by the attack of many forms of Botrytis.

Magee in 1933 (26) investigated chocolate spot disease of beans in New South Wales. He failed to isolate any bacterial or fungal pathogen, and experiments to determine whether the disease was of virus origin were also negative. During his experiments he found that the honeydew secreted by Aphis rumicis L. was toxic to bean shoots, producing reddish brown lesions. From this evidence he assumed that this honeydew was the causal agent of the disease in New South Wales.

#### DISTRIBUTION

Chocolate spot disease of broad beans Vicia faba L. caused Botrytis cinerea Pers. and B. fabae Sard. is widespread in North Western and non-

chernozem zones of the U.S.S.R. (46). In Israel is present all over the country (35). In Sudan the disease was present many years ago (37).

The disease was reported in Spain since 1929 (39, 50), Japan, Australia and Cyprus, all over Britain and Southern England and Burma (12, 13, 50). Scotland (16), New South Wales (26).

The disease is present in Florida, Georgia, New York, Alaska (18) and all over Canada (8). In 1945 was reported by Yu in China (49). The organism is common in the highlands of South America, in Guatemala and in most of places where the crop is grown.

#### IMPORTANCE

Widespread epidemic of chocolate spot were reported in Britain in 1920, 1926 and 1935 and localized outbreaks in 1923, 1924 and 1931. The epidemic of 1920 was responsible for 40-50 percent loss of the total bean crop in Southern England, while in individual fields losses of 75 percent or more were reported (29). During the epidemic of 1935 and 1944 a severe epidemic occurred all over England, yield were commonly said to be reduced by 50% and certainly some crops were rendered useless and had to be ploughed in (29, 50).

In Israel chocolate spot on broad beans caused heavy losses all over the country (35). In Sudan is the most serious diseases of broad beans caused by Botrytis cinerea Pers. and B. fabae Sard. (37).

#### NATURE OF LOSSES

'Chocolate spot' on leaves of field beans can be caused by either Botrytis cinerea or B. fabae. The spots caused by B. cinerea are inconspicuous compared with those caused by B. fabae, frequently only epidermal cells being affected whereas B. fabae nearly always causes

necrosis of the mesophyll. From the agricultural viewpoint, chocolate spot disease is that caused by B. fabae, since a bean crop even heavily infected by B. cinerea looks to be only insignificantly spotted, and little damage results from it (12, 13, 24).

Neither fungus sporulates on the chocolate spots, but only on leaves attacked 'aggressively', i.e. when the fungus brings about a general invasion of the leaf. Aggressive infection results from: 1) infection of damaged tissue; 2) a very heavy spotting of healthy leaves; 3) infected flowers falling on healthy leaves; and 4) senescence of lightly spotted leaves (24, 50).

Chocolate spot caused by B. fabae is first noticed in the field on isolated plants which are heavily spotted compared with those around them, infection presumably having originated on leaves damaged by frost or some other means.

Leaves and flowers of plants infected by Botrytis appear to play an important role in the build up of an epidemic. Not until the typical spotting had considerably increased, due to heavy sporulation of B. fabae on the lower leaves of the plants, did dead flowers start sporulating extensively with this fungus. Flowers cannot therefore be considered to play an important role in the critical period when chocolate spot suddenly builds up to epidemic proportions.

In a field of beans which has remained for some time only lightly spotted, there is always a time when the lower leaves start to become senescent and that is when the disease is likely to increase suddenly due to the fungus becoming aggressive in the lightly spotted leaves. Any set of conditions which induce premature senescence will encourage

the rapid development of chocolate spot providing climatic conditions are favorable for sporulation and infection. This would appear to account for the disease having been associated with apparently unrelated factors such as, for instance, deficiencies of either potassium or phosphorus. Well-balanced manuring and good drainage offer the best chance of bean crops withstanding serious, early attacks of chocolate spot (24, 50).

#### SYMPTOMATOLOGY

The symptoms range from a well-defined chocolate colored spotting of the leaflets and streaking of the stems to a "blight", where the lesions coalesce, causing partial defoliation, black ending and death of the plants (50).

On Leaves. The first form of infection unless very severe, does little damage, the fungus infect the leaf tissue and begin to secrete cell-wall degrading enzymes, which act upon the cell-wall to produce soluble derivatives. These stimulate the secretion of more enzymes and also provide carbon source for the further growth of the pathogen, (13) foliage produce subsequent to attack often being unaffected, in these attack, that appears on plants in the field as discrete, dispersed brown lesions, name "non-aggressive" stage. The lesions remain limited in size, with almost infinite variation in shape, color and surface. Discrete spots vary in size from 1 to 10 mm, and occur on both the upper and the lower surfaces but are frequently more numerous on the upper side (13, 17, 50), it is succeeded by a second 'aggressive' stage in which infected tissue is almost black, and the lesions cause extension leaf necrosis so that the plant itself may be killed in a relative short period (12).

On Stems. Stem lesions are usually superficial and may extend in streaks for several cm (50).

On Flowers. Petiole lesions are similar in appearance to those of the stem but deeper lying tissue are often involved, leading to complete collapse (50) dead flowers start sporulating extensively with these fungus (24).

On Pods and Seeds. Pods and seed coats may also show brownish blemishes (50).

#### HISTOLOGIC SYMPTOMS

In lesions of the leaflets, the epidermal layer may be totally disorganized leaving the palisade or the spongy parenchyma unaffected, or the disorganized zone may extend partly or entirely through the thickness of the lamina. Most of the affected cells collapse, the cytoplasm being marked by pigmented and granular and the cell walls swollen and often deep colored. Epidermal strips, unstained and mounted in water, were the most suitable preparations for the examination of the epidermal cells. The affected area is usually delimited by a zone of deeply pigmented cells. The nuclei are swollen and deep brown in color. Stomata guard cells and multicellular epidermal hairs are often deeply pigmented both inside and for some distance outside the area of the spot. Epidermal cells within the spot are presumably dead since, unlike normal cells, they are not plasmolysed by a 20 percent solution of sucrose. Just outside the area of the spot there is usually a slight pigmentation of the cells, the cytoplasm of which is more granular in appearance than normal. Such cells can, however, be plasmolysed and are therefore presumably living. Stem lesions present a similar histological appearance

but are usually superficial (50).

#### PHYSIOLOGICAL ASPECTS OF THE DISEASE

In the course of experimental investigation of the infection of bean shoots by B. cinerea it became increasingly evident that infection was of two types "aggressive" and "non-aggressive". The former causes blacking and death of part or the whole of the shoot system and is responsible for most of the loss caused by epidemic outbreaks of chocolate spot disease. The latter merely causes the death of localized areas of tissue, giving rise to discrete or coalesced lesions of the chocolate spot type (12,17,50). Immense variation of form occurs in this latter type of infection. Ikata cited by Wilson (50) suggested that similar variation in the lesions of "red-spot" disease of beans in Japan was due to atmospheric conditions and to variations in the carbohydrate content of the plants. He admitted, however, that further experimental work was required.

The cells of Vicia faba contain appreciable quantities of tyrosin and tyrosinase. The tyrosin is colorless, but when the cells are disorganized by the penetration of a fungus or other means, the tyrosinase brings about the oxidation of the tyrosin to melanin. According with Onslow cited by Wilson (50) the experimental oxidation of tyrosin by tyrosinase cause the production of a pink color deeping through red to black of melanin. The production of such a red pigment in the bean would account for the red or chocolate coloration which is so typical of many lesions on the plant. Any factor influencing that reaction, directly or indirectly, would bring about a change in size, shape and color of the lesions.



The extensive mycelium of B. fabae in the lesion must contribute directly to the increased respiratory rate shown to occur in the damaged tissue. The fungus can make little contribution to the increased rate in the peripheral green ring and none over 2 mm remote from the lesions. Therefore it is clear that the host tissue makes a marked metabolic response around the area of damage. Free polyphenoloxidase activity probably released from a latent enzyme in the leaf, was found in and around these lesions. Balasubramni et al. 1971 (2) suggested that the metabolic change in healthy parts of infected leaves is involved partly in synthesis of antifungal substances.

The cells invaded by the pathogen, and adjacent to it, are killed, possibly by the direct action of the cell-wall degrading enzymes and the latent phenolase of the cells becomes associated with soluble pectic substances which activate the phenolase, and this in turn acts upon phenols released by the death of the cells. The product of these reactions inactivate the pectic enzymes already secreted by the pathogen, and these interactions rapidly have a cumulative effect so that the growth of the lesions is prevented because cell-wall degrading enzymes are not produced in sufficient quantities to leave a balance which will continue to act upon unaffected leaf tissue.

Deverall in 1967 (10) found differences in diffusates of leaves containing B. fabae that were disappearance of sucrose, increase in levels of glucose and fructose, appearance of galacturonic acid after 6 hr, increasing to a maximum after 18 hr and fluctuations in amino acid levels with increase of aspartic and glutamic acid.

Purkayastha and Deverall in 1964 (36) shown an increase of anti-

fungal substances over the large lesions caused by B. fabae, a phytoalexin type of reaction was suggested. Later a substance named Weyerone was found and study (11, 14, 25, 27). Identification of the phytoalexin as weyerone was confirmed after treating the extracted acid with tritiated diazometane. Weyerone acid was not found on a few occasions when batches of leaves bearing large lesions were extracted after storage for several months at -20°C (25).

#### PATHOLOGICAL ASPECTS OF THE DISEASE

Last and Hamly in 1956 (23) developed a technique for measuring the infectivity of conidia of Botrytis fabae Sard. strain with one drop of a suspension of conidia in distilled water carried on the tip of the forefinger that was rubbed over the upper surface of a half-leaflet of a broad bean plant (cultiva 'Seville long pod') detached at round level and standing in a test tube of water. After inoculation the plants were kept under a unshaded perspex bell jar in a water saturated atmosphere at 20-25°C, conditions suitable for the development of lesions within 24 hr. At first the number of lesions were counted after 24 and 48 hr. but as there was rarely any significant difference between the two counts the second was discontinued.

Deverall and Wood 1961, (12) found that increasing the number of spores in the inoculation drops increase the number of lesions, and lesions were more readily produced on older than on younger leaves. The addition of glucose, a number of other carbohydrates and also sodium polypectate and pectin, increased the number and rate of the spread of lesions. Xylose caused a decrease, and a number of nitrogen compounds had little effect. The number and rate of spread of lesions was increased

if the surface of leaves were gently rubbed with a mild abrasive before inoculation. Calcium deficient plants were more susceptible than plants which had received normal nutrition. Deficiencies of other major elements had much less effect. A large proportion of the spores in inoculation drops germinated when these drops were on older leaves, when the drops contained glucose, and on leaf surfaces that had been gently abraded. Addition of xylose reduced the amount of germination and the fungus grew very poorly in liquid culture when this was the only carbon source. The addition of small quantities of glucose allowed the fungus to use xylose as efficiently as glucose. When single spore were used to inoculate leaves, very different results were obtained with leaves taken from different plants, but under some conditions single spores often induced lesion formation. B. fabae caused lesions more readily than did B. cinerea, in spite of the fact that a greater proportion of B. cinerea spores in inoculation drops germinated.

The same authors (13) studying the interactions between phenolase of host and pectic enzymes of the pathogen found that both organism B. fabae and B. cinerea, produce polygalacturonase abundantly in media containing pectic substances or soluble cellulose derivatives; only filtrates from cultures grown on polypectate had pectinesterase activity and even this was relatively low. B. cinerea filtrates from media containing cellulose had appreciable cellulase activity, but did not degrade insoluble wood cellulose; B. fabae filtrates were inactive in both respects. Cultures of both fungi on certain types of media readily macerated sections of bean stems and caused them to become brownish black in color.

Filtrates from cultures of both fungi had considerably lower polygalacturonase activities when leaf extracts were present in otherwise suitable media.

The latent phenolase present in water extracts of leaves was activated by polypectate, polygalacturonic acid, pectic and carboxymethylcellulose. Activation was most rapid near pH 4.5 and was negligible at pH 5.5 which was the optimum value for sodium dioctyl sulphosuccinate, one of the best activators so far described. Solutions of the polymers could be diluted with no great effect on activations.

Lesions are produced more readily on old than on young leaves, and extracts of old leaves discolour more rapidly than do extracts of young leaves when exposed to activated phenolase from bean leaves. This is because young leaves contain some system which retards the development of the colored products which are normally produced following the oxidation of phenols.

Water extracts of bean leaves inactivate the polygalacturonase of both fungi. The polygalacturonase of B. fabae differed from that of B. cinerea in being rapidly inactivated after filtrates had been diluted with water, and by being inactivated quickly by products formed after oxidation of dihydroxyphenylalanine, but was less affected when catechol was used; B. cinerea behaved in the opposite way.

Wastie in 1962 (4E) working with small water droplets containing known number of spores of Botrytis fabae and B. cinerea that were placed on detached leaves of broad beans plants, after 48-72 hr of incubation at high humidity, found the development of discrete chocolate-colored lesions in the areas under the drops was recorded as an all-or-none response.

Under experimental conditions used the  $ED_{50}$  for B. fabae was 3-4 spores, while that of B. cinerea was about 500. The slope of the dose-response curves suggest that lesion development is initiated by the independent action of individual spores rather than by the synergistic action of a number. Further evidence for this was afforded with B. fabae by the positive results of single-spore inoculations.

Deverall 1967 (10) studying the biochemical changes in infection droplets contained spores of Botrytis spp. incubated in the seed cavities of Vicia faba found that following B. fabae infection a biologically inactive ultraviolet (u.v.) absorbing substance appears in high yield in place of the antifungal substance formed following B. cinerea infections.

In 1969 Deverall and Vessey (11) studying the role of the phytoalexin found in controlling lesion development in leaves of broad bean attacked by chocolate spot found that the phytoalexin was formed in leaves by apparently healthy cells in advance of hyphae of either B. fabae or B. cinerea, and in response to physical injury. Concentrations of this phytoalexin around deep lesions caused by B. fabae were completely fungistatic B. fabae caused apparent degradation of phytoalexin in lesions and removed phytoalexin from solutions in vitro much more readily than did B. cinerea. The lower sensitivity to the phytoalexin and the possibly related greater ability to metabolize the phytoalexin, are major factors in the greater pathogenicity of B. fabae than B. cinerea. The same properties largely explain the ability of B. fabae to cause the so called "aggressive" phase of the chocolate-spot disease under some conditions. Detection of phytoalexin in apparently healthy tissue around

deep lesions caused by B. fabae and in mechanically damaged tissue suggest that phytoalexin production by Vicia is a general response of cells in advance of damage.

The observation on mycelial growth and cellular Browning made by Balasubramani et al. 1971 (2), suggest that with rare exceptions broad bean cells react rapidly to the presence of approach of Botrytis hypha and undergo necrosis. Their studies show that the green peripheral rings of tissue cut from around lesions caused by B. fabae are almost free of hypha, but the host tissue makes a marked metabolic response around the area of damage. Its high activity in dark brown lesions in broad bean, the desintegration of these lesions and the detection of monogalacturonic acid in infection droplets over these lesions do not support the idea that inactivation of the enzymes by oxidized phenols is an effective means of limiting the fungus in vivo. Further evidence was obtained in their work of the ready inactivation of polygalacturonase in vitro. Possibly rate of production exceeds rate of inactivation in vivo. Inactivation in vitro may be dependent upon dilution of the enzyme, so inactivation may not occur at all in vivo.

Fawcett et al. 1971 (14) had expected to find a decrease in Weyerone (the phytoalexin) levels in broad bean leaves after infection with B. fabae as a consequence of a conversion of Weyerone to weyerone acid. It therefore came as a surprise to discover that B. fabae, the pathogen which causes the aggressive lesion of chocolate spot disease, should also lead to such a large increase in weyerone. After 4 days weyerone reach the maximum level and then begins to fall. This fact could be associated with a rapid conversion of weyerone to the more antifungal weyerone acid and hence be an important factor in disease resistance.

Mansfield and Siddowson 1973 (27) found that both B. fabae and B. cinerea were able to metabolize wyerone acid following germination of conidia. This suggest that the lower toxicity of wyerone acid to conidia of B. fabae than of B. cinerea is caused by a lower sensitivity of B. fabae to the phytoalexin and not to a greater ability of B. fabae to degrade wyerone acid. The work provides evidence that the ability to metabolize phytoalexin may be an important characteristic of some phytopathogenic fungi.

#### SIGNS

When conditions are sufficiently humid the blackened tissue becomes covered with spores; this rarely happened in the non-aggressive phase (12).

#### ETIOLOGY

In 1923 Paine and Lacey, cited by Wilson 1937 (50) recognized the presence of a infection by Botrytis sp. that frequently followed attacks of chocolate spot, that he thought was caused by Bacillus lathyri.

In 1929 Rodriguez Sardiña (39) give the following description of the new specie named Botrytis fabae Rz. Sardiña.

"Céspedes formando una capa poco compacta y de color francamente castaño claro, y más tarde blanquecina, nunca tupida y gris como en el grupo Cinerea. Micelio en el interior de la matriz, hialino al principio, después castaño más o menos obscuro, pluriarticulado (cédulas de 12, 70  $\mu$  de longitud), con hifas de 4,9 - 15,1  $\mu$  de diámetro. Las ramas conidíferas salen por los estomas, y a medida que se van desarrollando

estomas, y a medida que se van desarrolland los conidióforos, va constituyéndose un estroma de color castaño obscuro, sobre el que quedan implantados los sucesivos aparatos conidíferos que se forman. Conidióforos de 162-351  $\mu$  de longitud, pardo oscuros en la base y empalideciéndose hacia arriba hasta ser hialinos en sus últimas ramificaciones, de contenido finamente granuloso (sólo en algunas regiones, tanto de las partes castañas como de las que son hialinas, es como espumoso), erguidos unos ensanchados en la base en forma cónica más o menos globosa, y otros no; con 1-3 tabiques en su tronco principal y 3-4 ramas primarias; estas últimas tienen 1-3 tabiques y, o no se ramifican o rara vez lo hacen en dos ramas terminales; las terminaciones de estas ramas últimas se hallan algo dilatadas en forma esférica o ligeramente piriforme, y en ellas están insertos los conidios mediante cortos esterigmatos; las cabezuelas de conidios que se forman alrededor de esta última ramificación, tienen de 39 a 63  $\mu$  de diámetro. Conidios ovoideos, a veces algo irregulares y frecuentemente son la cicatriz de inserción del estigma bien marcada; en grandes masas presentan un color manifiestamente castaño claro y aún en preparación montada en agua y observada a un aumento de 200 por 1, ponen un ligero color amarillento; sus dimensiones son: 15,2 - 24,3  $\mu$  de largo (la mayor parte de 20,0-23,1  $\mu$ ), y 10,9 -18,2  $\mu$  de ancho (la mayoría de 13,9 - 16,4  $\mu$ ), valores medios de 200 esporas 20,6 x 14,6  $\mu$ . Esclerosios no vistos en plantas atacadas naturalmente, ni tampoco en las inoculadas experimentalmente, pero sí en los cultivos... Los de los cultivos en agar de infusión de habas con glucosa (1 x 100) son semi-esféricos o semi-elipsoideos, a veces algo irregulares, de color negro de humo, mates, con la superficie lisa o algo tuberculosita y de 1,0 - 3,6 x 0,9 - 2,2 x 0,4 -2,0 milímetros, media de



25 esclerocios = 1,7 x 1,5 x 0,9 mm<sup>3</sup>.

"Hab.: sobre hojas y tallos vivos de Vicia faba L., produciendo una marchitez y podredumbre en plantas de cultivos".

Pathogenicity. The pathogenicity of Botrytis fabae was established by Rodriguez Sardiñas in several experiments (39) from this time it is well established the two different types of Botrytis, B. cinerea Pers. as a "non-aggressive" type, causing discrete or coalesced chocolate colored lesion and B. fabae as an "aggressive" type causing blackening and death of part of the whole of the shoot system (2, 3, 5, 10, 12, 13, 17, 23, 24, 27, 48, 50).

The genera Botrytis belongs to the family Moniliaceae, order Moniliales of the Deuteromycetes (Fungi Imperfecti) (7).

#### LIFE HISTORY

Primary cycle. Winter broadbean debris, according with Ogilve and Munro 1946, cited by Moore and Leach 1968 (30) are considered the most important reservoir for infection by chocolate spot (B. fabae Sard.). Usually B. fabae first becomes established in young frost damaged winter beans on which it can spore freely providing foci of infection and inducing spotting on surrounding plants. These spots, however do not spore until the leaves bearing them senesce and the fungus become aggressive, thus starting an epidemic.

Secondary cycle. Leaf of plants adjoining the foci of infection can become heavily spotted and succumb to aggressive infection. Dead broadbean flowers are frequently covered with Botrytis spores and these have previously been thought to play an important part in the etiology of chocolate spot disease because they fall on to the bean leaves and

cause aggressive infection resulting in a further output of spores. Collection of such flowers were made from several fields (Leach 1955) (24) and sporulation test clearly showed that in broadbean fields showing only a little chocolate spot the flowers were almost entirely infected by B. cinerea.

Retarding the senescence of the lower leaves should therefore retard the epidemic spread; the senescent leaves were more frequently attacked aggressively. Several substances or closer plant spacing could retard or reduce leaf senescence (30).

The initial stages of the diseases caused by both fungi are similar and they result in the formation on the leaf, more rarely on other parts of the plant, the lesions already described. If the plants are growing well, in conditions which are not particularly humid, the lesions remain small and although the fungus can be recovered from the diseased tissue, it does not sporulate on the surface. When conditions are particularly suitable for the pathogen, the lesions spread rapidly and the "spot" effect is lost because the pathogen colonized large areas of leaf tissue in a matter of a few days. The diseased tissue becomes black in color and the fungus often sporulate profusely on the dead leaf surface. This later stage of the disease, for which the term chocolate spot is not really appropriate, is called "aggressive phase" and is more commonly caused by B. fabae than by B. cinerea (13).

When Buxton et al. (5) treated spores of B. fabae with ultra violet irradiations observed that the pathogenicity decreased, as assessed by counts of local lesions or pustules. The infectivity was lost more rapidly than the ability to form colonies on agar. Ultraviolet radiations change to spores was mitigated by exposure to daylight after irradiation.

The extend of such photoreactivation was the same whether the spores were on the host plant or in vitro. Ultravioleta irradiation of leaves before inoculation increased the number of lesions. Rubbing leaves with Celite before inoculation also increase the number of lesions. Re-taining ultravioleta irradiated broadbeans plants in day light or darkness after inoculation with unradiated spores of B. fabae did not significant-ly alter the lesions number.

Last 1960 (22) studying the longevity of conidia of B. fabae found that equal percentages of conidia from 10 and 40 days old cultures germinated, but only those from the younger culture infected the plant bean. Whereas infectivity fell by 90% within 25 days of culturing germination did not fall to this extent until 85 days. Ageing conidia seem to contain adequate reserves for germination but insufficient to meet the demands for infection. Tests were therefore made to see whether infectivity could be restored to conidia from cultures 20-30 days old by supplementing their nutrition. At a concentration of 45% citric acid and sucrose increased the number of lesions from 9.5 in the water control to 21.0 and 35.5 per 1/2 leaflet respectively. In tests with a range of sugars, results varied between experiments but sucrose consistently increased infectivity the most, followed by glucose mannose and maltose; the pentose sugars, arabinose and xylose, had no effect. The infectivity of conidia from young cultures, 13 and 19 days old, was no significantly increase by galactose, but the number of lesions produced by cultures 33 and 41 days old were increased by factor X5.0 and X3.6 respectively. Similarly, sucrose increased the infectivity of old cultures more than that of young cultures; number of lesions given by conidia from cultures 5, 24 and 35 days old were increased by X1.7, X6.0, X4.3 respectively. The lost of

infectivity, as measured by number of lesions is not clearly attributable to the mechanical inability of germinating conidia to penetrate, as was shown by Blackman and Welsford in 1916, or to the inability to spread subsequently and disrupt the host. That the loss of infectivity with increasing age is at least partially attributable to the inability to penetrate was suggested by the results of his experiments with "Celite". Conidia from cultures 11 and 19 days old gave 49 and 8 lesions on intact leaves respectively, but when leaves were abraded so that penetration was easy, approximately equal numbers of lesions were developed.

#### EPIDEMIOLOGY

The most important environmental factor affecting the prevalence and severity of chocolate spot in broad beans appear to be relative humidity (R.H.) (17, 29, 46). The most critical period with respect to this factor in its influence upon the disease is at blossoming time, and the latter stage of plant development.

In 1937 Wilson (50) studied four physical factors of the environment, air temperature, air humidity, air movement and light on the chocolate spot disease of broad beans.

Air temperature. From a series of three experiments he concluded that the maximum temperature for infection is close to 30°C. No infection was obtained at 32°C, the optimum temperature was clearly in the vicinity of 20°C. At this temperature infection occurred in 8-12 hr, while at 5°C a similar intensity of infection was obtained only after 3-4 days. Infection occurs at 1°C after 7 days. The minimum temperature for infection, therefore, lies between +1 and -1°C.

Air humidity. Rodríguez Sardiñas in 1931 cited by Wilson (50) found that the minimum percentage relative humidity for infection of beans by B. Fabae Sard. was 84-85 percent.

Wilson 1937 (50) found that no infection occur under 85 percent of R.H. but at 100% of R.H. infection occur from 28- to 5°C of temperature. At 90% of R.H. a considerable infection occur at 10 to 20°C with the optimum at 15°C.

Tupenevich and Kotova 1970 in Russia found that high R.H. and temperature of 20-23°C favor infection. In wet, warm years epiphytotic may occur (46).

From reports since 1917, Moore 1949 (29) found that in general epidemics of chocolate spot occur only about one year in six, and that in addition, there are local epidemics in different places. The indications are that chocolate spot becomes epidemic only when severe late spring frost are followed by warm, wet weather in June or July, but he recognized that epidemiological research is required before the accumulated observations on the disease can be used to the best advantage.

In 8 of the 12 years investigated by Hogg 1956 (17), there was a close relation between occurrence of the disease and the frequency of near-saturation hours (95% of R.H.). Only in one year no apparent relationship was established. Three of the years showed at least a moderately close relation. The results of his studies indicate fairly conclusively that the incidence of chocolate spot is related to the frequency of near saturation hours. Disease indice possibly depends on a threshold value of near-saturation hours, but he recognized that further work would be needed to establish this. It is possible that the onset of

chocolate spot, like potato blight is predisposed by the occurrence of a certain period when the relative humidity is continuously above a given limit.

Air movement. At lower speed, the minimum R.H. needed for initial infection at 15°C dropped from 90 to 80 percent (50).

Light. Wilson 1937 (50) found that under 20°C as constant temperature and in the dark and diffuse daylight, no difference in rate or intensity of infection on broad bean are in the plants.

The optimum external environmental conditions for chocolate spot are: a) the presence of large number of spores on the foliage, b) rain to establish a water film, c) a high humidity and further rain to maintain the film over a considerable period of time, d) little or no sun and wind to increase evaporation and e) an air temperature close to 20°C.

### CONTROL

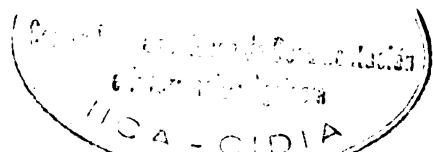
#### EXCLUSION

The common occurrence of Botrytis cinerea Pers. in almost all countries where broad bean is grown, preclude the possibility of successful exclusion of it from fields or gardens. The polyphagous condition of this organism contributes to the fact that is almost impracticable the exclusion of chocolate spot from any field.

Tupenevich and Kotova 1970 (46) recommended seed treatment with thiram, in a pre-sowing treatment with molybdenum, in order to reduce the initial infection.

#### ELIMINATION (Erradication)

Crop rotation, with non-susceptible host, in areas continuously



devoted to broad bean are recommended due the build up of inoculum in debrays. In general, chocolate spot can be minimized by growing the plants under conditions as favorable as possible, by allowing free circulation of air so that the atmosphere does not become too humid and by the destruction of dead and moribund tissue (3).

Newhook 1951, (32) found that saprophytic antagonism is responsible for some of the natural control of B. cinerea in the field. Control of B. cinerea on Epilobium sp., Ranunculus repens, R. flammula and Lamium amplexicaule was obtained by prior inoculation of killed tissues with soil suspensions.

Strains of Bacillus, Pseudomonas and Chromobacterium isolated from dead leaves of lettuce seedling overwintering in the open, have been proved to be antagonistic in various degrees to B. cinerea on nutrient agar on wounded detached lettuce leaves and on wounded leaves of seedlings at temperatures ranging from 4 to 25°C under conditions of high moisture and humidity (31, 32).

Wood 1951 (51) found a substantial control of B. cinerea when young potted plants in frames were sprayed with suspensions of selected antagonists in 1.0% glucose solution. Any application of antagonists is likely to have at the most a temporary effect and since moribund and dead tissue appears throughout the winter, repeated application would be necessary, in contrast fungicides having a more persistent effect require fewer applications. It seems probable that the development of cultural methods encouraging continued and rapid colonization of dead tissue by saprophytes and the stimulation of their growth by addition of suitable materials would be more practical and successful than the direct applications of selected antagonists.

The antagonism of the bacteria investigated, however, was largely due to antibiotic substances quite independently of the effect of the high pH. B. cinerea on nutrient agar and on lettuce leaves produced a strongly acid reaction. This was capable of neutralizing the alkaline reaction due to most bacterial activity only if the latter was not already well established. Many strains of bacteria cause lysis of young active Botrytis mycelium and germ tubes. In association with many bacteria, B. cinerea has exhibited features such as stimulation or depression of sporulation, continuation of aerial growth with inhibition of lateral spread of mycelium, visicular distortion of hyphae and germination of spores while still attached to the conidiophores. Some spores of the test strain of B. cinerea used gave rise to new strains more resistant to antagonism than the original.

#### PROTECTION

Bordeaux mixture (10-10-100) was found to be more satisfactory than lime sulphur, given complete control, in the test made by Wilson 1937 (50) on potted plants that were treated with a standard spore suspension and placed under optimum conditions for infection. Test on field plots were of little value, as conditions suitable for epidemic attack did not rise.

Tupenevich and Kotova 1970 (46) recommended to spray with captan or zineb, both 0.5%.

Fawcett et al. 1957 (15) found that the degree of infection of plants treated with griseofulving was rarely significantly different from that of controls, when testing seedling of 10-14 day inoculated with the conidia of B. fabae.



No satisfactory method of control has been found. The chances of the disease assuming epidemic proportions are, however, lessened if care is taken to correct fertility deficiency and improving drainage. Time of planting could be a good way to avoid winter damage, but are more liable to insect infestation (50).

#### IMMUNIZATION (PLANT RESISTANCE)

I was not able to find recent reports of resistant varieties used in this country. The only report is from Russia that mention 2 resistant varieties 'Purple pod' and 'Hmelnickie' as resistant to Botrytis sp. (40).

#### OTHER DISEASES ON *Vicia faba* L.

Rust, Uromyces fabae (Persoon) De Bary (6, 18, 19, 20, 21, 28, 35, 37, 49)

Powdery Mildew, Erysiphe polygoni De Candolle (34, 37, 41, 43, 49)

Blight Ascochyta fabae Spegazzini (8, 9, 40, 45, 49)

Leaf spot, Cercospora fabae Fautrey (8, 49)

Foot rot, Fusarium oenaceum var. fabae Yu (49)

Wilt, Fusarium oxysporum f. sp. fabae Yu & Fang (49)

Root rot, Rhizoctonia solani (1, 18)

Bacterial disease, Pseudomonas fabae (4)

Bacterial Blight, Xanthomonas phaseoli (E.F. Sen.) Dows (18)

Curly top virus, Ruga verrucosans Carsner & Bennett (18)

Mosaic virus (18)

Spotted wilt virus, Lethum australiense Holmes (18)

Root rot, Fusarium culmorum (W. G. Sun) Sacc. (18, 49)

Root rot, F. trichothecoides (18)

Knot nematode, Meloidogyne spp. (18)

Leaf spot, Mycosphaerella sp. (18)

Root rot, Phymatotrichum omnivorum (Shear) Dug (18, 49)

Root rot, (Damping-off) Phytium debaryanum Hesse (18, 49)

Stem rot Sclerotinia, sclerotiorum (Lib.) De Bary (18, 49)

Souther blight, Sclerotium rolfsii Sacc. (18, 28)

Leaf and stem spot, Alternaria tenuis (18, 49)

Powdery mildew Leveillula taurica (37, 41)

"Mildew del haba" Perenospora vicia De Bary (28)

Broad Bean Mottle Virus, Smith (28)

Other diseases associated with Vicia faba are:

Ascochyta pisi Lib (8, 28)

Ascochyta viciae Libert (18, 33, 49)

Botryobasidium rolfsii (Saccardo) Venkatayan (49)

Colletotrichum viciae Dearhess and Overholts (49)

Stemphytium botryosum Wallroth (49)

Thanatephorus cocumeris (Frank) Donk (49)

Thielaviopsis basicola (Ber, and Bro.) Fer. (49)

Sclerotinia trifoliorum (33)

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