Etiological and pathogenicity studies on the bacterial pod spot of rape

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The bacterial pod spot of rape first reported froni southern Alberta in 1973 has since been found in central Alberta and appears to be quite widespread. The brown lesions produced on the pods by some isolates of the pathogen may be bordered by chlorotic haloes. All varieties of Polish rape ***(Brassica campestris)*** and Argentine rape ***(B. napus)*** tested have proved susceptible. Wounding in various ways seems to predispose rape to infection. The causal bacterium is seed borne and has been identified as a strain of ***Pseudomonas syringae.*** Five isolates of the bacterium from rape reacted identically in biochemical and physiological tests and were very similar to one of ***P. syringae*** frorn lilac that produced symptoms on inoculated pods of rape resembling those of the pod spot isolates.

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Signalée pour la premiere fois dans le sud de l'Alberta en 1973, la tache des siliques du colza a depuis été observee dans le centre de la province et semble très repandue. Il peut arriver que les lesions brunes produites sur les siliques par certains isolats du microbe soient entourees de halos chlorotiques. Toutes les variétés de navette ***(Brassica campestris)*** et de colza (B. ***napus)*** testées se sont révélées sensibles. Les blessures semblent prédisposer la culture à l'infection. La bacterie responsable, transmise par la semence, semblerait être une souche de ***Pseudomonas syringae.*** Cinq isolats de la bacterie provenant du colza ont reagi de la même façon à des tests biochimiques et physiologiques et ressemblaient Btroitement, apres inoculation sur des siliques de colza, a un isolat du lilas manifestant des symptômes qui ressemblent à ceux des isolats de la tache des siliques.

Bacterial pod spot was first reported from southern Alberta affecting crops of Span rape on two farms near Rockyford in 1973 (3). The causal organism, a species of ***Pseudomonas,*** infects its host most readily through wounds. Further studies on the identity and pathogenicity of the causal bacterium are reported here.

Though as yet no thorough survey of the distribution of bacterial pod spot has been made in Alberta we know now that it is not confined to southern Alberta. Judging from specimens received and from field examinations, we believe that the disease is widespread and that it may develop wherever and whenever conditions favorable for its development occur. The disease has been found at a number of points in central Alberta including the Edmonton and Edgerton areas; there, and no doubt in other parts of Alberta besides the southern part, conditions often favor the incidence of the disease end the spread of the causal bacterium. While rape has been the principal host found affected in the field there is reason to suspect that other hosts, including several horticultural plants, may be affected.

Materials and methods

The pathogen was isolated from affected rape pods and seeds grown in pure culture on nutrient agar, potato sucrose agar, and other media, where it produced grayish white, shiny colonies. Dried exudates of the bacterium that developed on lesions were scraped off with a sterile scalpel, dispersed in sterile water, and the suspension streaked on agar plates; colonies were allowed to develop for a few days, as suggested by Dowson (2). If no exudates were present lesions were cut out with a sterile scalpel and allowed to soak overnight in a few ml of sterile water, which was then streaked on agar plates; the same procedure was followed with whole seeds suspected of being infected or infested. Single colony isolates were established on nutrient agar.

Isolates of the pathogen from two main sources have been studied. The original ones were obtained in 1973 from infected pods of Span rape ***(Brassica campestris*** L.) from two farmers' fields at Rockyford in southern Alberta. The isolates from the second source were obtained in 1975 from lightly frosted seed of Tower rape (***B. napus*** L.)grown at Edgerton in the east central part of Alberta.

Plants of several varieties of B. ***campestris*** and ***B. napus*** were grown in the greenhouse from seed kindly supplied by Dr. Z.P. Kondra, University of Alberta. Fresh well developed green pods from these plants were then inoculated by a standardized procedure to determine the relative pathogenicity of different isolates of the pathogen and the comparative reactions of different rape varieties to them. The use of detached freshly harvested green pods was more convenient and more rapid than a similar procedure using attached pods and appeared to give similar results. Inoculum was applied to small wounds made in the pod wall with a sterile steel needle dipped in an artifical culture of the pathogen on nutrient or other agar. Two punctures for inoculation purposes,

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one toward each end of the pod, were made and a third one in the center between inoculation points was made with a sterile needle to serve as a wounded check (3). Another method of inoculation that proved effective consisted of the immersion of pods similarly wounded in a suspension of the bacterium in sterile water for several hours. Following inoculation the pods were immediately placed on the surface of sterile water agar in petri plates and incubated for 1-2 weeks at 20°C or at room temperature.

Observations and results

Isolates of the pathogen from different sources have been found to be similar in many of their characters. This constancy is quite remarkable in view of the different environments to which these isolates were exposed. But as might be expected this pathogen like many others exhibits variability in several characters; particularly important are those of pathogenicity and toxin production.

Variability in pathogenicity of rape isolates of ***Pseudomonas*** sp. has been observed in our studies not only when rape has been inoculated but also when other hosts, e.g. bean, have been inoculated with rape isolates. Though high pathogenicity was more commonly found in isolates from central than in those from southern Alberta many more isolates need to be studied before conclusions on distribution can be drawn. Toxin production, indicated by the formation of pale chlorotic haloes around brown pod lesions, occurred with certain isolates of ***Pseudomonas,*** but with others haloes were absent or poorly developed. Variability in halo production by ***Pseudomonas*** species has been observed in other bacterial plant diseases, e.g. of beans caused by ***Pseudomonas phaseolicola*** and of oats caused by ***Pseudomonas coronafaciens.*** In bacterial pod spot of rape we so far have observed halo production more commonly in rape inoculated with isolates of ***Pseudomonas*** from central Alberta (Fig. 1A) than with those from southern Alberta (Fig. 1B).

Attention was drawn in a previous article (3) to the fact that pre-disposition of the host by wounding is important in encouraging infection by the bacterium. This has been supported by further observations, especially of factors operating in the field. Two which appear worthy of special attention and which were not mentioned specifically in our previous paper are frost and insect wounding. Both probably play significant roles in the development of bacterial pod spot of rape in Alberta and no doubt account in part for the occurrence and severity of the disease.

The comparative pathogenicity of isolates of ***Pseudomonas*** was tested on varieties of rape using green pods of approximately the same stage of maturity. These pods were wound inoculated and incubated as described, and severity of infection was judged by the size and character of the lesions. All isolates were treated in the same way. Similar results were obtained for all varieties inoculated with the isolates tested. The isolates from southern Alberta were in general less pathogenic than those from east central Alberta as was judged by the severity of infection produced. For example, on pods of Span rape, isolates from Span pods from southern Alberta produced less severe symptoms than isolates from Torch seed from central Alberta. There was also less tendency for the southern isolates to produce haloes around their lesions than was true of isolates from east central Alberta. This probably indicates differences in toxin production. It cannot be said however from the limited observations made to date that pathogenicity is directly related to toxin production. It would seem however that varietal differences in both exist but especially in respect to pathogenicity the differences so far observed have not been particularly sharp and consistent. It appears from preliminary tests however that certain differential non-cruciferous hosts may be found useful in distinguishing isolates with respect to pathogenicity.

The bacterial pod spot disease of rape was first reported in 1973 affecting fields of Span rape, a Polish variety. Soon after, it was found affecting another Polish variety, Torch, in the field. It seemed important to determine if the disease was confined to Polish varieties or if it might affect Argentine varieties as well. A preliminary test in which the Polish variety Torch and the Argentine variety Midas were inoculated with two isolates of ***Pseudomonas*** from southern Alberta showed the two varieties to be equally susceptible; both isolates produced definite dark lesions on pods of both varieties.

In a second test four Polish varieties and four Argentine varieties representing different types of rape grown in Alberta were inoculated with a highly pathogenic seed isolate of ***Pseudomonas*** from Tower rape grown in central Alberta. The results of this test (Table 1) showed that all eight varieties are susceptible and that the Argentine varieties are as susceptible as the Polish varieties. Unfortunately no marked resistance was detected in any variety.

Studies undertaken to determine the specific identity of the causal organism consisted mainly of biochemical and physiological tests. In addition a few comparisons with isolates of ***Pseudomonas*** affecting noncruciferous plants were made.

The bacterial isolates under study all proved to be fluorescent pseudomonads and to be oxidase negative and arginine dehydrolase negative (Table 2). Hence according to the eighth edition of Bergey's Manual (1) they belong to the "Pseudomonas syringae" group. Using methods outlined in papers by Hildebrand et al. (4), Lelliott et al. (5), Misaghi et al. (6), and Stanier et al. (8), five isolates from rape were shown to be identical. An isolate of ***Pseudomonas*** from lilac reacted similarly to those from rape in all tests except gelatin liquefaction, for which rape isolates were negative and the lilac isolate positive (Table 2). It should be noted that such variability

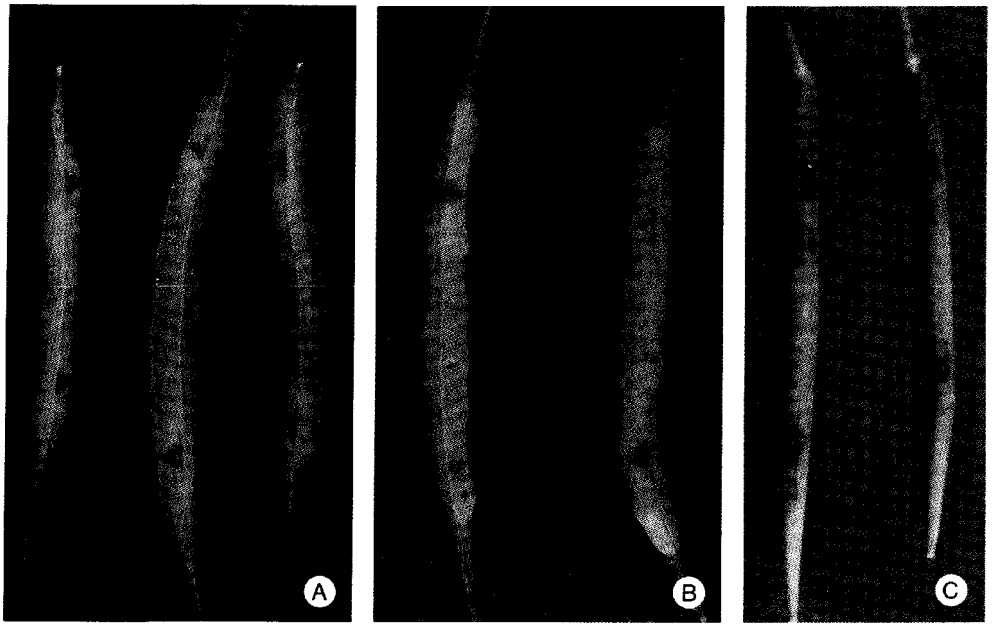

Figure 1. Detached pods of rape inoculated with isolates of *Pseudomonas syringae*. A) Lesions with chlorotic haloes on pods of Midas rape inoculated with an isolate from central Alberta. B) Lesions lacking haloes on pods of Midas rape inoculated with an isolate from southern Alberta. C) Definite dark lesions on pods of Span rape inoculated with an isolate from lilac. Pods were inoculated through a wound near each end; a wound near the center of each pod was not inoculated.

Table 1. Reactions of eight rape varieties to a seed isolate of *Pseudomonas syringae* from Tower rape

Species and variety	Severity on inoculated pods
Brassica campestris (Polish rape)	
Span	light
Torch	medium
R–500	heavy
Arlo	medium
Brassica napus (Argentine rape)	
Midas	heavy
Tower	medium
Target	medium
Nugget	medium

Table 2. Biochemical and physiological comparisons of five isolates of *Pseudomonas* from rape with an Alberta isolate of *Pseudomonas syringae* from lilac

Biochemicat and physiological tests	*Pseudomonas* isolates from rape					*Pseudomonas syringae*
	OS73–9 BII	OS73–9 XXC	0875–51 I	0875–51 VI	0875–51 VIII	DP74–98, Lilac
Nitrite reduction	–	–	–	–	–	–
Arginine dihydrolase	–	–	–	–	–	–
Oxidase	–	–	–	–	–	–
Fluorescent pigment	+	+	+	+	+	+
Reducing substances (sucrose)	+	+	+	+	+	+
Gelatin liquefaction	–	–	–	–	–	+
Indole production	–	–	–	–	–	–
Levan	+	+	+	+	+	+
Acid from lactose	–	–	–	–	–	–
Acid from maltose	–	–	–	–	–	–
Acid from glucose (aerobically)	+	+	+	+	+	+
Acid from glucose (anaerobically)	–	–	–	–	–	–
Pectate liquefaction	–	–	–	–	–	–
Pathogenicity on green rape pods	+	+	+	+	+	+

is commonly found among isolates of P. *syringae.* On the basis of these tests therefore the rape isolates are not distinguishable from the P. *syringae* isolate from lilac.

As noted above, an isolate from lilac that we consider to be *Pseudomonas syringae* proved pathogenic to rape, affecting green rape pods as severely as several rape isolates. It is noteworthy that van Hall originally isolated P. *syringae* from lilac in 1902 (1). Another host which has been found to harbour a *Pseudomonas* capable of attacking rape, though less severely than the lilac isolate, is the raspberry. Among leguminous plants, beans have been reported as susceptible to P. *syringae.* Patel et al. for instance have found *P. syringae* to be the cause of bacterial brown spot of beans in Wisconsin (7). The symptoms of this disease are in several respects similar to those of the disease of rape which we have called bacterial pod spot. Moreover certain of our rape isolates have infected green bean pods producing definite brown lesions on them.

It is concluded from the results reported here that strains of *P. syringae* can cause bacterial pod spot of rape and that several noncruciferous plants may serve as additional hosts. Though the five rape isolates studied here appear identical, it is recognized that considerable variability in the causal agent of this disease exists and

that further work may uncover other strains of the pathogen.

Acknowledgments

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