

Identification of Soft-Rot *Erwinia* Associated with Blackleg of Potato in Arizona

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ABSTRACT

Pathogenicity and physiological tests indicated that *Erwinia carotovora* var. *carotovora* (sensu Graham) is the primary causal agent associated with blackleg of potato in Arizona.

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Additional key words: *Erwinia carotovora* var. *atroseptica*.

Blackleg of potato, a bacterial disease, occurs wherever potatoes are grown (1,9,10). In Arizona, the disease is responsible for loss of stand, increased numbers of small, weak plants, and death of older plants. Yields are generally reduced by 1.0 to 10%, but individual fields may sustain losses up to 40%. Plants exhibiting blackleg symptoms, typical of those reportedly caused by *Erwinia carotovora* var. *atroseptica* (van Hall) Dye (9), are the most frequently encountered. However, some diseased plants only appear wilted with a brown to black discoloration of the vascular tissue and/or pith. This can extend up the stem approximately 2 to 10 mm from a soft-rotted seed piece (13). Because the two types of symptoms were observed, the identity of the causal bacterium associated with typical and atypical symptoms was investigated.

Stems from 96 and 15 individual potato plants (*Solanum tuberosum* L. 'Norgold Russett') exhibiting typical and atypical symptoms, respectively, were collected throughout the growing season (March through June) from different commercial fields. Diseased pieces of tissue from each stem, dissected from the advancing margin of the rot, were individually suspended in sterile water and aliquots of the suspension streaked onto nutrient agar (Difco) and m-Endo LES agar (Difco) containing 0.5% sucrose. After 48-72 hours of incubation at 25 C, typical *Erwinia*-like colonies were easily selected and transferred to calcium polypectate gel (5). Pectate hydrolyzers were plated again onto nutrient agar, and their purity verified. Subsequently, single colonies were transferred to yeast-dextrose-carbonate (YDC) (3) slants on which they were maintained throughout the study.

Isolates which were gram-negative, oxidase-negative, and fermented glucose within 48-72 hours were classified as soft-rot *Erwinia* spp. Physiological tests for varietal differentiation, conducted according to Graham (6), were: production of indole, acid from maltose, and α -methylglucoside, and the production of reducing substances from sucrose. Growth at 37 C was determined

following the method of Dye (3).

Pathogenicity of each isolate was determined by puncturing stems of 6-week-old potato plants (cultivar Norgold Russett) with moist toothpicks smeared with cells from 24-hour cultures grown on YDC medium. Toothpicks were left in place. Appropriate control plants were punctured with sterile toothpicks. All plants were incubated at 18.3 C (6,7) in a moist chamber for 2-3 days. All pathogenicity and physiological tests were repeated at least twice.

In total, 111 isolates from as many plants, representing 96 from plants exhibiting typical blackleg symptoms and 15 from plants exhibiting atypical blackleg symptoms, were obtained. All isolates produced stem blackening and wilting, typical of blackleg symptoms, within 24 and 48 hr after inoculation. Control plants remained healthy. Results of pathogenicity and physiological tests, along with the results of Graham (6) and Dye (4), for varietal differentiation of *E. carotovora* var. *atroseptica* are presented in Table 1. Since no isolates produced indole, none were considered to be *Erwinia carotovora* variety *chrysanthemi* (6).

Characterization of bacterial isolates showed that only 4.5% and 2.7% possessed all the physiological features of *E. carotovora* var. *atroseptica* sensu Graham (6) and Dye (4), respectively. However, 58.5% and 30.6% of the isolates corresponded to *E. carotovora* var. *carotovora* sensu Graham (6) and Dye (4), respectively. The remainder of the *E. carotovora* isolates could not be identified to variety. Isolates recovered from plants which exhibited atypical symptoms, showed the same range of variation as those which exhibited typical blackleg symptoms.

European researchers have demonstrated that *E. carotovora* var. *atroseptica* is the primary (if not the sole) causal bacterium associated with blackleg of potato (7,8,10,11). Results of our study, however, indicate that the causal bacterium of blackleg in Arizona is primarily *E. carotovora* var. *carotovora* (sensu Graham (6)). This variety has also been occasionally associated with blackleg in Colorado (M. D. Harrison, Colorado State University, *personal communication*) and in Israel (M. C. M. Perombelon, Scottish Hort. Res. Inst., *personal communication*). Since approximately 50% of our isolates were incapable of growth at 37 C, it appears that the growing conditions in Arizona have not selected strains adapted to high temperatures.

Our *E. carotovora* var. *carotovora* isolates, and the isolates not identified to variety, induced blackleg of potato at 18.3 C, whereas only *E. carotovora* var. *atroseptica* is reportedly capable of doing so (6,7). Therefore, either more than one variety of *E. carotovora* must be responsible for blackleg in diverse geographical areas and/or the existing criteria employed for varietal differentiation are inadequate due to the range of variation within the species.

Although *E. carotovora* var. *atroseptica* is primarily tuber-borne, and does not overwinter in field soils (8, 9, 11, 12), *E. carotovora* var. *carotovora* can be tuber-borne (11) and also soilborne (14). The origin of the effective inoculum for inciting blackleg in Arizona, particularly in heavily cropped agricultural areas, needs clarification. At the present time, however, this is difficult

TABLE I. Results of comparative pathogenicity and physiological tests used to identify *Erwinia carotovora* var. *atroseptica* from 111 isolates of *Erwinia carotovora* obtained from blackleg-infected potato plants

Criteria	Isolates positive ^a	Isolates expected positive	
	(%)	Graham ^b (%)	Dye ^c (%)
Blackleg, pathogenicity test at 18.3 C	100	100	Not tested
Reducing substances from sucrose	22.5	98	80-100
Acid from maltose	19.8	87	80-100
Acid from α -methylglucoside	19.8	94	80-100
No growth at 37 C	46.8	variable	80-100
Indole production	0	0	0-20

^aPercentage of 111 isolates of *E. carotovora*, obtained from blackleg infected plants, which gave a positive reaction.

^bPercentage of isolates expected to give positive reactions for identification of *E. carotovora* var. *atroseptica* according to Graham (6).

^cPercentage of isolates expected to give positive reactions for identification of *E. carotovora* var. *atroseptica* according to Dye (4).

to determine since production of blackleg from naturally contaminated tubers planted in either sterile or field soil has not been achieved with any degree of regularity (10, authors *unpublished*). Further, existing selective media or baiting techniques employed to detect soft-rotting *Erwinia* spp. in fallow field soils are insensitive to populations generally below 100 cells/g of soil (2,10,14). Until proven otherwise, lack of recovery from soil using these techniques does not preclude their absence. More sensitive techniques for isolating blackleg-producing varieties of *E. carotovora* from Arizona soils are currently under investigation.

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