Pathogenicity of Strains of *Pseudomonas syringae* pv. *savastanoi* and Their Indoleacetic Acid-Deficient Mutants on Olive and Oleander

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ABSTRACT

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Previous investigations have shown that indoleacetic acid (IAA) production by strains of *Pseudomonas syringae* pv. *savastanoi* (pv. *savastanoi*) from oleander is necessary for formation of galls on oleander. The present study compared the pathogenicity on olive and oleander plants of wild-type and indoleacetic acid (IAA)-deficient (Iaa^-) mutant strains of pv. *savastanoi* from olive, oleander, and privet. All wild-type strains from olive, oleander, and privet induced galls on olive. Only wild-type isolates from oleander were tumorigenic on oleander. An exception was observed with isolate EW1017 from olive which also caused galls on oleander; however, this strain resembled strains from oleander in that IAA genes were plasmidborne. Iaa^- mutants of the strains from olive and privet did not induce galls on olive and oleander. Iaa^- mutants of strains from oleander produced no symptoms on oleander plants, but induced atypical galls on olive plants. The role of bacterial production of IAA in gall formation on the olive and oleander knot diseases was thus confirmed.

Additional key words: olive knot, phytohormones, plasmids.

Olive knot, which is incited by *Pseudomonas syringae* pv. *savastanoi* (Smith) Young et al (hereafter referred to as pv. *savastanoi*), is a common disease of olive (*Olea europaea* L.) and is characterized by the formation of galls on stems and, less frequently, on other parts of the plant. A similar disease occurs on oleander (*Nerium oleander* L.) (17) and privet (*Ligustrum japonicum* Thunb.) (3).

Pathovar *savastanoi* synthesizes indole-3-acetic acid (IAA) (1,2) and substances with cytokininlike activity (13). Smidt and Kosuge (10) demonstrated that IAA-deficient (Iaa^-) mutants of pv. *savastanoi* from oleander could not induce gall formation on oleander. In strains from oleander, the enzymes for production of IAA are encoded by genes borne on the pIAA plasmid (5). At least two forms of pIAA have been found: the 52 kilobase (kb) pIAA1 in California strains 2009 and 2015 from oleander (4), and the 72 kb pIAA2 in strains PB205 and PB213 from oleander in Italy (6). In strains from olive and privet, however, the genes for IAA production are chromosomal (6). Iaa^- mutants can be easily isolated from most strains (Comai et al, *unpublished*), regardless of the location of IAA genes. Conflicting reports have appeared on the pathogenicity of strains from olive on oleander (3,7,9,11,12,14,15) and on the utilization of sucrose by isolates from different hosts (3,16).

The objectives of this study were to determine whether IAA is involved in symptom development on olive by using Iaa^- mutants of strains of pv. *savastanoi* from olive, privet, and oleander, and to compare host responses to isolates of pv. *savastanoi* from olive, privet, and oleander.

MATERIALS AND METHODS

Bacterial isolates. Eleven wild-type isolates of pv. *savastanoi* were used: PB230, PB231, PB207, and EW1017 from olive; PB204, PB205, and PB213 from oleander; and PB217, PB218, PB209, and PB215 from privet. Isolate EW1017 was isolated in California; all

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others were isolated in Apulia, Italy, and maintained by the Istituto di Patologia Vegetale, Bari, Italy. All were progeny of single cells and were pathogenic on their homologous host with the exception of PB204 which, originally pathogenic on oleander, spontaneously lost pathogenicity and capacity for IAA production during repeated subculture. Other Iaa$^-$ mutants used in these experiments were isolated by selection for α-methyltryptophan resistance by the procedure of Smidt and Kosuge (10). Selection for α-methyltryptophan resistance yields Iaa$^-$ derivatives in most strains of pv. *savastanoi*. Loss of IAA production results in resistance to α-methyltryptophan, probably through increased tryptophan concentration in the cells (Smidt and Kosuge, *unpublished*).

Pathogenicity tests. Since knot symptoms develop more rapidly on young stem tissue, olive and oleander plants were severely pruned to stimulate development of new growth as side branches. Wild-type isolates and their Iaa$^-$ mutants were inoculated in 1-yr-old branches of olive (unknown cultivar) and in stems of 3-mo-old shoots of oleander. The bacterial inocula were grown for 2 days on ANS (nutrient broth, 0.8%; sucrose, 5.0%; and agar, 2.0%) slants and were applied with a wooden applicator directly to wounds

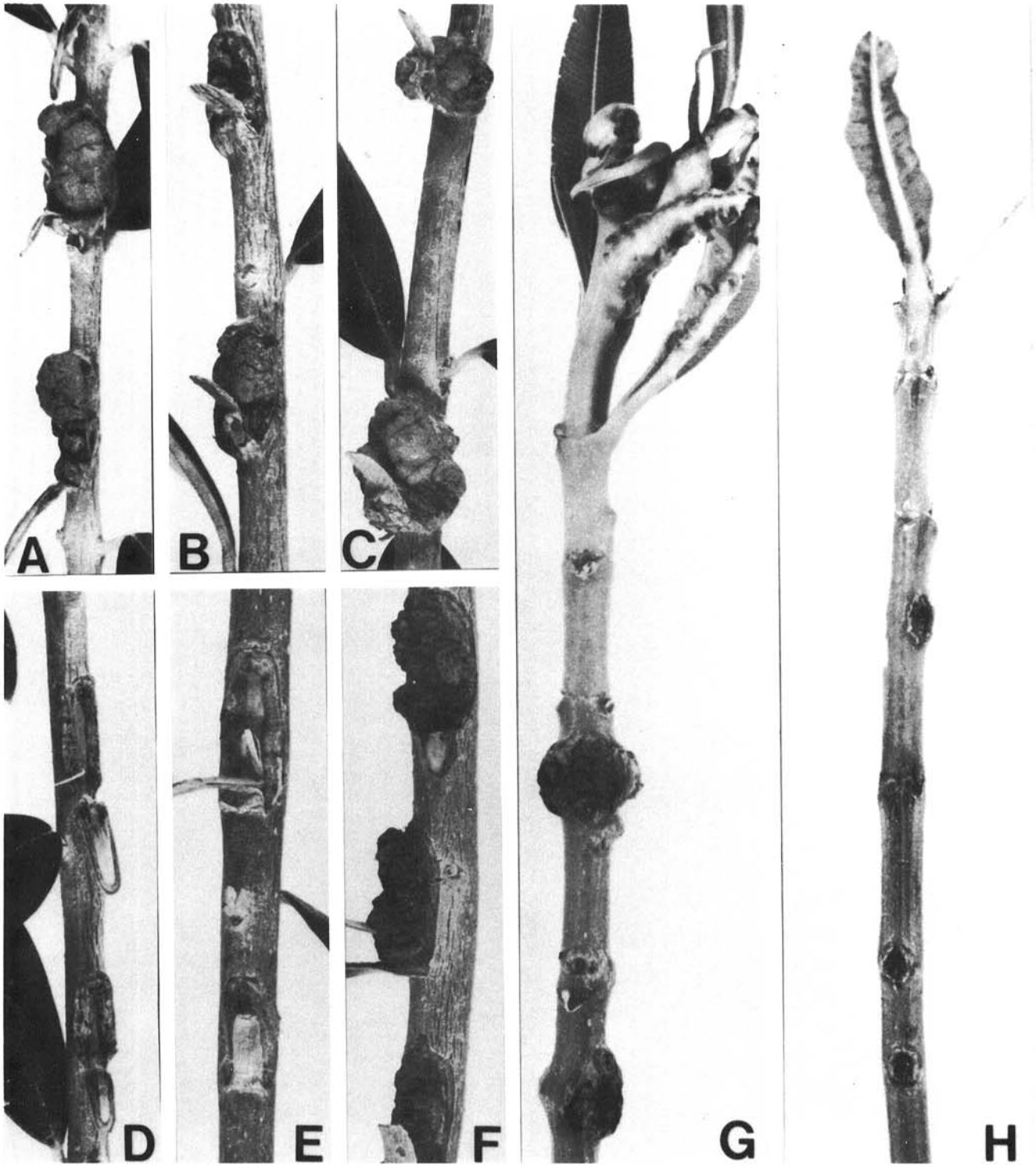

Fig. 1. Typical symptom development on young stems of olive (**A to F**) and oleander (**G and H**) inoculated with wild-type *P. syringae* pv. *savastanoi* isolates (A, B, C, and G) and their indoleacetic acid-deficient (Iaa$^-$) mutants (D, E, F, and H). **A and D,** Isolate PB230 from olive; **B and E,** isolate PB218 from privet; **C and F,** isolate PB205 from oleander; **G and H,** isolate PB213 from oleander. Control plants are not shown since they displayed symptoms similar to those on plants D and E.

made in the bark of olive stems. Approximately 10^8 colony-forming units (CFU) were applied to each wound. To inoculate oleander, bacterial cells were suspended in saline (10^9 CFU/ml) and 0.1 ml of a bacterial suspension was injected with a 0.455-mm (25-gauge) needle in the second internode. For controls, olive and oleander plants were similarly injected with distilled water. Inoculation sites were covered with parafilm for 24 hr to prevent drying. A total of four inoculations was made for each strain; two inoculations per branch were made on two separate branches of a single olive plant or one inoculation per branch was made on four separate branches of individual oleander plants.

All the inoculations were made during the third week of April 1981, at Davis, CA. A second set of inoculations in May 1982 confirmed the results reported here. Plants were held in a greenhouse at ambient light and temperature (25 ± 2 C) and observed for symptom development up to 60 days after inoculation.

Analytical procedures. Plasmids were isolated and separated by agarose gel electrophoresis by the procedure described previously (4). The Salkowski assay technique (10) was used for analyses of indoleacetic acid in cell-free filtrates from cultures grown in King's medium (8).

RESULTS

Pathogenicity tests. All wild-type isolates of pv. *savastanoi* induced typical symptoms on olive plants (Table 1, Fig. 1). Symptoms were first discernible 10–15 days after the inoculation as two separate masses of new tissue proliferating at both edges of the injury. These tissue masses proliferated progressively until they joined on the surface of the cut area to form a mature gall (Fig. 1). No major differences were observed in the appearance of the galls produced among strains of the three host isolation groups, except for rate of development and ultimate size of the mature knots (Table 1). Knots did not form on olive plants inoculated with the Iaa$^-$ mutants of strains of pv. *savastanoi* from olive and privet plants or on control plants; instead, only a small proliferation of the tissue was observed at the edges of the inoculation site. Sections of such structures showed water-soaked areas from which avirulent bacteria could be isolated. On the contrary, the Iaa$^-$ mutants of

TABLE 1. Gall formation on olive and oleander by wild-type (Iaa$^+$) and indoleacetic acid-deficient (Iaa$^-$) mutants of *P. syringae* pv. *savastanoi*

Host of Origin	Isolate	Host response to pv. *savastanoi* phenotype:[a]			
		Iaa$^+$ on:		Iaa$^-$ on:	
		Olive	Oleander	Olive	Oleander
Olive	EW1017	+ (324)	+[b]	–	–
	PB207[c]	+ (273)	–	NT	NT
	PB230	+ (161)	–	–	–
	PB231	+ (254)	–	–	–
Oleander	PB213	+ (158)	+	[+][d] (141)	–
	PB205	+ (109)	+	[+] (107)	–
	PB204[e]	NT[f]	NT	[+] (68)	–
Privet	PB217	+ (187)	–	–	–
	PB215	+ (149)	–	–	–
	PB218	+ (128)	–	–	–
	PB209	+ (152)	–	–	–

[a] Numbers in parentheses are the average fresh weights (in milligrams) of galls 60 days after the inoculation. Responses of host: + = gall-like tissue proliferations; – = absence of tissue proliferations (see Fig. 1).
[b] The morphology of galls induced on oleander plants by strain EW1017 prevented accurate weight measurements.
[c] No Iaa$^-$ mutants of PB207 have been yet isolated since this strain has high resistance to α-methyltryptophan.
[d] [+] indicates atypical growths induced on olive by oleander Iaa$^-$ mutants.
[e] No Iaa$^+$ strain of PB204 is available.
[f] NT = Not tested.

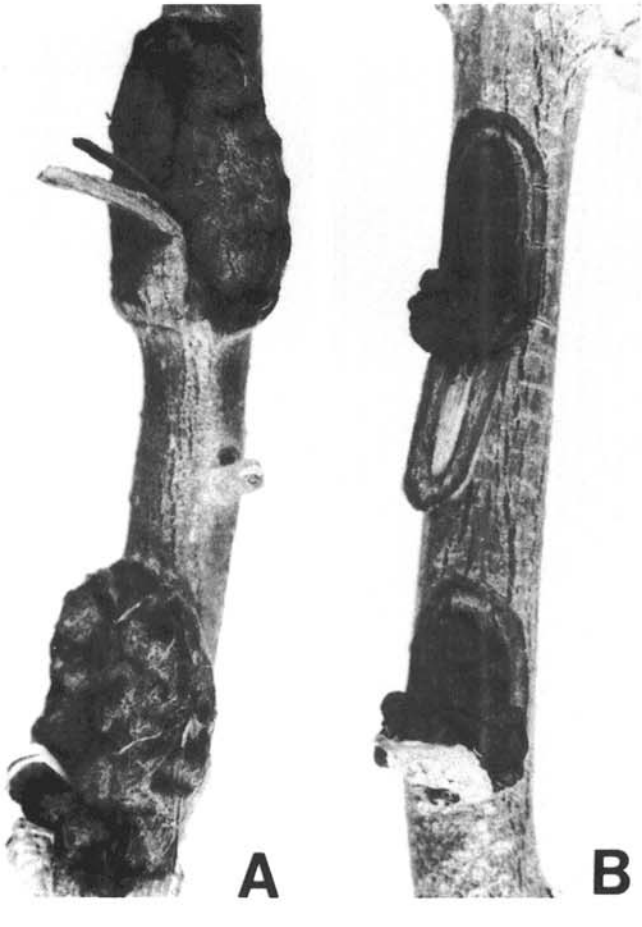

Fig. 2. Symptoms induced on olive stems after inoculation with **A,** wild-type olive isolate PB230 and **B,** indoleacetic acid-deficient (Iaa$^-$) mutant of an oleander isolate PB205 (B). Note the peculiar morphology induced by Iaa$^-$ mutant of the oleander strain PB205 in comparison to the typical "olive knot."

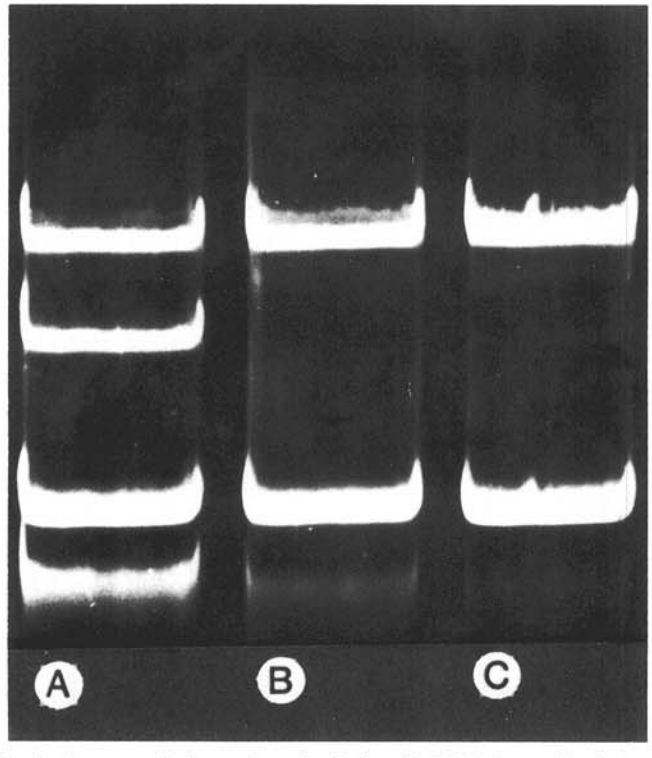

Fig. 3. Agarose gel electrophoresis of plasmid DNA from olive isolate EW1017. **A,** Wild-type; **B and C,** indoleacetic acid-deficient (Iaa$^-$) mutants characterized by the lack of a 70-kilobase (kb) pair plasmid encoding IAA production. The largest plasmid exhibited a mobility corresponding to 96 kb, the smallest to 50 kb. The plasmid patterns of other isolates used are shown elsewhere (6).

isolates PB205, PB213, and PB204 from oleander were avirulent on oleander and induced abnormal tissue proliferation on olive which appeared greener, softer, and more corrugated than knots produced by parental isolates (Fig. 2).

On oleander, only wild-type strains PB205 and PB213 from oleander and the atypical strain EW1017 from olive induced typical "knot" symptoms including galls at the site of inoculation and occasional secondary tumors and curling of the apical leaves (Fig. 1). All oleander plants inoculated with the strains of pv. *savastanoi* from privet and the remaining strains from olive were symptomless. None of the Iaa$^-$ mutants induced galls on oleander. The atypical characteristics of isolate EW1017 were further revealed by plasmid analysis which showed that its genes for IAA production occur on a 70 kb plasmid (Fig. 3), unlike other isolates from olive which have IAA genes on the chromosome. Mutants of EW1017 cured of the plasmid were avirulent on oleander and were Iaa$^-$ (Fig. 3, Table 1).

Sixty days after inoculation, bacteria were reisolated from infected olive tissues and compared with their original cultures for morphological characteristics on ANS and King's B (8) media as well as for plasmid composition. Each had retained characteristics of the corresponding strain initially used for inoculation.

DISCUSSION

It was fortuitous that selection for α-methyltryptophan resistance yielded Iaa$^-$ derivatives of strains 2009 and 2015 (10) which are cured of pIAA1 (4). When the plasmid was reintroduced into the pIAA1-less derivative, 2009-3, IAA synthesis was restored (4). Further, a 2.7 kb fragment of pIAA1 bearing gene *iaaM*, the determinant for tryptophan monooxygenase, was cloned in *Escherichia coli* SK1592 (5). Thus, the work reported here provides further evidence on the role of IAA as a factor of pathogenicity in the interaction between pv. *savastanoi* and oleander and olive (4,5). All IAA producers tested, regardless of host origin, were virulent on olive. Iaa$^-$ mutant strains from olive and privet failed to induce symptoms on olive. The Iaa$^-$ mutants of strains from oleander were symptomless on oleander, but elicited atypical overgrowths on olive plants. All Iaa$^-$ mutants of the three strains of oleander from Italy induced these atypical galls on olive. Contamination of the inoculated plants by pathogenic isolates from olive is unlikely; none of the control plants developed knots. Furthermore, galls with similar characteristics were not observed in any of the other inoculation sites and only cells characteristic of the inoculated strains were reisolated from these overgrowths and identified by their plasmid DNA profiles and colony morphologies on ANS and King's B media.

The basis for the unexpected behavior of Iaa$^-$ mutants of strains from oleander (PB204, PB205, and PB213) on olive plants (Fig. 2) is not clear. However, it could be due to response of the plants to production of elevated levels of cytokinins by these mutants (D. Regier, R. Morris, and T. Kosuge, *unpublished*) (13).

On oleander plants, only the homologous wild-type Iaa$^+$ strains and an aberrant strain, EW1017 from olive, induced symptoms. The remaining wild-type strains from olive and all the Iaa$^-$ mutants were symptomless on oleander.

Strain EW1017 from olive differs from typical isolates from olive in two major traits: it induces knots on oleander plants and bears the genes for IAA production on a 70 kb plasmid (Fig. 3). Further, a fragment of pIAA1 containing the monooxygenase gene hybridizes with the 70 kb plasmid, but not with chromosomal DNA of strain EW1017 (L. Comai and T. Kosuge, *unpublished*). Isolates of EW1017 cured of the plasmid are Iaa$^-$ and avirulent on oleander.

Since strains of pv. *savastanoi* from oleander are generally pathogenic on olive, it is conceivable they spread under natural conditions from oleander and cause knots on olive plants. If such is the case, one can expect to isolate from olive knots bacteria with characteristics of strains from oleander, particularly if the olive plants grow close to infected oleander plants. One such example may be isolate strain EW1017, which has physiological and pathological characteristics closer to those from oleander than to those from olive yet is capable of causing knots on both species of plants. It remains to be determined if the extrachromosomal location of the IAA genes is consistently linked with the broader host range of strains from oleander.

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