Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 4368–4372, June 1990
Biochemistry

A nontransformable *Triticum monococcum* monocotyledonous culture produces the potent *Agrobacterium vir*-inducing compound ethyl ferulate

(signal molecule/plant cell transformation)

ERIC MESSENS, RUDY DEKEYSER, AND SCOTT E. STACHEL*

Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000 Gent, Belgium

Communicated by Marc Van Montagu, February 20, 1990

ABSTRACT **Exudates of dicotyledonous plants contain specific phenolic signal molecules, such as acetosyringone, which serve as potent inducers for the expression of the virulence (*vir*) regulon of the phytopathogen *Agrobacterium tumefaciens*. This induction activates the *Agrobacterium* T-DNA transfer process to initiate the genetic transformation of target plant cells. Wounded and metabolically active plant cells are particularly susceptible to *Agrobacterium* infection, and these cells specifically produce *vir*-inducing molecules. Most monocotyledonous, as opposed to dicotyledonous, species are resistant to *Agrobacterium* transformation. One hypothesis for this resistance is that nonsusceptible monocotyledonous cells fail to produce *vir* signal molecules and, thus, are not recognized by *Agrobacterium* as transformation targets. Here we demonstrate that monocotyledonous cells make such molecules, and, furthermore, we purify the inducer produced by a *Triticum monococcum* suspension culture that is resistant to *Agrobacterium* infection. This molecule is shown to correspond to ethyl ferulate [$C_{12}H_{14}O_4$; 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid ethyl ester], to be more active for *vir* induction at low concentrations than acetosyringone, and to be produced in quantities giving significant levels of induction. Thus, at least for the wheat cell line used in this study, monocotyledonous resistance to *Agrobacterium* transformation must result from a block to a step of the T-DNA transfer process subsequent to *vir* induction.**

The soil phytopathogen *Agrobacterium tumefaciens* genetically transforms plant cells to cause the neoplastic disease crown gall (1). In the bacterium–plant cell interaction a specific element, the transferred DNA (T-DNA), is mobilized from a donor bacterium to a susceptible recipient plant cell, localized to the plant cell nucleus, and stabilized by chromosomal integration. Expression of T-DNA genes in the plant cell leads to its transformation, which provides *Agrobacterium* with specialized nutrients. The T-DNA, which is carried in the bacterium on the large-tumor-inducing (Ti) plasmid, does not encode the functions that mediate its transfer; these functions are provided by a separate 35-kilobase-pair (kbp) region of the Ti plasmid, the virulence (*vir*) region. This region carries six essential complementation groups (*virA, virB, virC, virD, virE,* and *virG*), which comprise the *vir* regulon (2). In vegetative bacteria, only *virA* and *virG*, the *vir*-positive-regulatory genes are expressed; conversely, when *Agrobacterium* is cultivated with wounded plant cells, expression of the complete *vir* regulon becomes induced to a high level (2, 3). This transcriptional activation initiates the T-DNA transfer events (3, 4) and limits them as to when they can lead to plant transformation (5).

In nature, only plant cells that have been wounded are targets of T-DNA transfer (6). Furthermore, dicotyledonous and not monocotyledonous plant species are generally susceptible to *Agrobacterium* (7, 8). Plant cell wounding likely provides *Agrobacterium* access to the plant cell membrane, a requirement for the close cell–cell contact essential for T-DNA transfer. Thus, *Agrobacterium* must be able to recognize and respond to wounded and metabolically active plant cells. *Agrobacterium* accomplishes this by coupling the activation of its *vir* regulon to specific diffusible compounds secreted by wounded plant cells (3). We isolated and identified (5) two compounds present in the wound exudates of metabolically active *Nicotiana tabacum* cells, acetosyringone (4-acetyl-2,6-dimethoxyphenol) and α-hydroxyacetosyringone [4-(2-hydroxyacetyl)-2,6-dimethoxyphenol], which function as highly specific and potent inducers of *Agrobacterium vir* expression (5). These compounds, which are produced by root, leaf, and callus tissues (3, 5), further serve as potent chemoattractants for *Agrobacterium* (9) and also transcriptionally activate several non-*vir* loci located on both the Ti plasmid and the *Agrobacterium* chromosome (10).

The *Agrobacterium*–plant interaction has practical importance as a vector system for transferring DNA of interest to plant cells (for review, see ref. 11). T-DNA transfer has been used widely to alter plant cells genetically and to probe the requirements of plant gene expression. A goal of such work is the improvement of important crop plants by means of genetic engineering. However, the major crop species, the monocotyledonous cereal plants, have generally proven refractile to *Agrobacterium* infection (8). Thus, effort has been directed toward both developing alternative methods to effect monocotyledonous transformation and understanding why most monocotyledons resist *Agrobacterium*.

Monocotyledonous resistance could result from several factors, including (*i*) failure of *Agrobacterium* to recognize monocotyledonous cell-membrane components, (*ii*) failure of T-DNA integration in monocotyledonous cells, or (*iii*) inability of wounded monocotyledonous cells to proliferate after infection. The simplest explanation for this block is that monocotyledonous cells are not initially recognized by *Agrobacterium* as transformation targets because they fail to produce *vir*-inducing compounds in their exudates or produce them in quantities limiting for significant induction. This hypothesis is supported by the observation that the monocotyledon *Dioscorea bulbifera* is only transformed by agrobacteria preinduced with dicotyledonous wound exudate (12). However, two groups have reported that nontransformable monocotyledons do, in fact, make *vir*-inducing compounds. Okker *et al.* (13) reported that monocotyledons

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Ti, tumor inducing; T-DNA, Ti plasmid DNA sequence stably transferred to plant; *vir*, virulence gene.

*Present address: Department of Human Genetics, University of Utah, Salt Lake City, UT 84108.

produce a high-molecular-weight compound that induces *vir* expression in *Escherichia coli* in the absence of the essential *vir*-regulatory machinery. In light of subsequent studies on the *vir*-induction process, this increase probably corresponded to nonspecific stimulation of general metabolism, not *vir* induction. Using the standard *Agrobacterium vir*-induction assay, Usami *et al.* reported results on the ability of monocotyledon cells to produce *vir* signal molecules. First these workers (14) indicated that monocotyledons fail to make such compounds. In a later study they reported (15) that wheat and oat cells produce a *vir*-inducing factor that greatly differs from the dicotyledon molecules in being hydrophilic and several thousand daltons in size, although its structural identity was not determined. Thus, the questions of whether the exudates of nonsusceptible monocotyledons contain a *vir*-inducing activity and what its molecular nature is are unresolved.

We have assayed the exudates of several monocotyledon culture lines for *vir*-inducing activity and show here that both susceptible and nonsusceptible monocotyledon species produce such compounds. Furthermore, we isolated and purified the active compound produced by a wheat suspension culture line resistant to *Agrobacterium* infection. The wheat *vir* inducer is structurally identified as ethyl ferulate, shown to be more active at low concentrations than the potent tobacco *vir* inducer, acetosyringone, and to be produced in quantities sufficient for significant *vir* induction. Thus, at least for wheat, the block to *Agrobacterium* transformability is from a defect other than the nonproduction of *vir*-inducing compounds. We discuss possible causes of this barrier.

MATERIALS AND METHODS

Preparation of Plant Cell Exudates. Suspension culture cell lines of five monocotyledonous species (*Zea mays, Triticum monococcum, Hordeum vulgare, Oryza sativa*, and *Asparagus officinalis*) were grown in plant growth media for 5–7 days. Filtered growth media were subsequently tested for *vir*-inducing activity. To prepare the wheat cell exudate for purification of inducing activity, *T. monococcum* suspension cell culture was grown as described by Lörz *et al.* (16). After 5- to 7-day growth, 2.7 liters of spent culture media were recovered, clarified by centrifugation at 9500 × *g*, filtered through Whatman GF/C, and extracted twice with chloroform. The chloroform phase was dried and concentrated, and the final residue of the wheat cell exudate was dissolved in 15 ml of 10% aqueous acetonitrile containing 0.1% F_3CCOOH.

Purification and Identification of *vir*-Inducing Compound from Wheat Cell Exudate. Reversed-phase chromatography was done as described (5). Initially, 1% of the chloroform-extracted wheat cell exudate was separated on a Pep reversed-phase chromatography prepacked column (5 × 50 mm; HR5/5, Pharmacia). For preparative separations, one-third portions of the extract were fractionated on a Pep reversed-phase chromatography 10 × 100 mm column (HR10/10, Pharmacia). Columns were preequilibrated with aqueous 0.1% F_3CCOOH and eluted at either 0.7 ml/min (analytical) or 2.8 ml/min (preparative) with increasing (1% per min) proportions of acetonitrile up to 60% in aqueous 0.1% F_3CCOOH. One-minute fractions were collected over 60 min from both analytical and preparative runs monitored at 214 nm. Each fraction was assayed for biological activity, and fractions containing the *vir*-inducing activity were pooled. This material was purified to homogeneity by additional separation on the HR10/10 column with a 15–25% acetonitrile gradient in aqueous 0.1% F_3CCOOH at 0.25% per min.

Assay for *vir*-Inducing Activity. *Vir*-inducing activity was assayed using *Agrobacterium* tester strains carrying *vir*::*lac* fusion genes (3, 17); *vir* induction in these strains is quantitatively assessed by measuring β-galactosidase activity, the *lacZ* gene product. Entire analytical fractions or 100-μl aliquots from the preparative fractions were lyophilized and resuspended in 1.5 ml of Murashige and Skoog (MS) medium supplemented with 3% sucrose, 0.018% K_2HPO_4, 0.01% inositol, and 0.0001% biotin, pH 5.5 (5). Each fraction in the supplemented MS was inoculated with either the *Agrobacterium virB*::*lacZ* strain A348(pSM30) or the *virE*::*lacZ* strain A348(pSM358) to a final OD_{600} of 0.1 per ml and incubated 10–15 hr at 25°C and shaking at 200 rpm. β-Galactosidase activity is expressed as specific units per bacterial cell. The relative *vir*-inducing activities of the purified wheat-specific compound, synthetic ethyl ferulate, and commercial acetosyringone were compared by dissolving each in the supplemented MS medium to a final absorbance of 1.0 per ml at their respective λ_{max}, measured against supplemented MS; each solution was then serially diluted and tested for *vir*-inducing activity.

Chemical Analysis. GC/MS was done with a Finnegan 4000 instrument interfaced with an Incos data system. For GC, 0.1-μl samples in 0.1 mM chloroform were directly injected onto a 0.25 mm × 20 m OV-1 column and eluted at 155°C at 3.5 ml of helium per min. A portion of the purified lyophilized wheat *vir*-inducing compound was derivatized by treatment with 0.1 ml of *N,O*-bis(trimethylsilyl)trifluoroacetamide (All-tech) in 0.1 ml of acetonitrile and subjected to GC/MS analysis.

Preparation of Ethyl Ferulate. Ferulic acid (4-hydroxy-3-methoxycinnamic acid) was dried over phosphorus pentoxide under vacuum, and 1 mmol was dissolved in 45 ml of anhydrous acetonitrile containing 0.1 mmol of F_3CCOOH and 4 mmol of anhydrous ethanol. A 2.5 molar excess of dicyclohexylcarbodiimide (DCC), dissolved in 5 ml of acetonitrile, was added, and the mixture was kept at 40°C for 90 min with constant stirring, diluted with 150 ml of water, and left for 3 hr at room temperature. The aqueous filtrate was twice extracted with 150 ml of chloroform. The chloroform extract was washed with 10% sodium chloride in water followed by a water wash and dried overnight on excess anhydrous sodium sulfate. Chloroform was evaporated, and the remaining residue was dissolved in 2 ml of anhydrous acetonitrile at 40°C. Petroleum ether (boiling point, 40°–60°C) was added until a faint turbidity was seen. After standing overnight at 4°C, a colorless oil of ethyl ferulate separated out. The products formed at the different reaction steps were followed by separation on a Pep reversed-phase chromatography HR 5/5 analytical column and GC/MS analysis.

RESULTS

Monocotyledonous Cell Lines Produce *Agrobacterium vir*-Inducing Compounds. Exudates of monocotyledonous cell lines were assayed for *vir*-inducing activity. Table 1 shows that different monocotyledon cell lines produce from none (barley) to low (maize, rice), moderate (asparagus), or high (wheat) levels of *vir* inducers. Although the variable levels of *vir*-inducing activity among different species of plant cell

Table 1. *Vir*-inducing activity in conditioned medium from monocotyledonous suspension cell cultures

Family	Species	Units*	*vir*-inducing activity
Gramineae	Maize	50–70	Low
Gramineae	Wheat	550–1100	High
Gramineae	Barley	20–30	None
Gramineae	Rice	50–70	Low
Liliaceae	Asparagus	100–150	Medium
		20	Background

*Units per bacterial cell when using the *virB*::*lacZ* tester strain A348(pSM30).

lines is presently without explanation, the results show that monocotyledon cell exudates can effect *vir* gene activation.

Of the monocotyledons tested, only asparagus is readily sensitive to *Agrobacterium* transformation (18, 19). Potentially, the general lack of monocotyledon transformability could result from monocotyledons producing less active *vir*-inducing molecules, such that only subthreshold levels of induction are stimulated. To test this hypothesis, monocotyledon *vir* inducer was purified from the exudate of the wheat suspension culture as this material gave the highest levels of *vir* induction (Table 1). We used the procedure previously used to isolate tobacco *vir* inducers: the wheat activity was partitioned into chloroform, and the subsequent chloroform extract was fractionated by reversed-phase chromatography. The elution/activity profile in Fig. 1 shows that the *vir*-inducing activity elutes as a single peak at 18% acetonitrile, which corresponds to 8% of the total material in the chloroform extract absorbing at 214 nm. The *vir*-inducing activity in the material in the center of this peak was purified by further reversed-phase chromatography to homogeneity. Approximately 125 μg of *vir*-inducing compound was isolated from 2.7 liters of the wheat-conditioned medium; because this corresponds to roughly 50% of total peak material, we estimate that the *vir*-inducing compound was present at $\approx$90 μg per liter in the starting exudate.

Identification of Wheat-Specific *vir*-Inducing Compound. Fig. 2*A* shows the mass spectrum of the wheat-specific *vir*-inducing compound, determined to be 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid ethyl ester. The most abundant ion in the spectrum occurs at m/z 222, consistent with the molecular formula $C_{12}H_{14}O_4$. The next most abundant ion, at m/z 177, is consistent with the loss of an ethoxy ($C_2H_5O\cdot$) radical involving an α-cleavage reaction at a carbonyl group. The ion at m/z 194 corresponds to the loss of carbon monoxide (CO) or ethylene (C_2H_4); the loss of carbon monoxide is characteristic of phenolic hydroxyl groups, and the loss of ethylene occurs through a McLafferty rearrangement (20). The ion at m/z 145 is explained by the combined

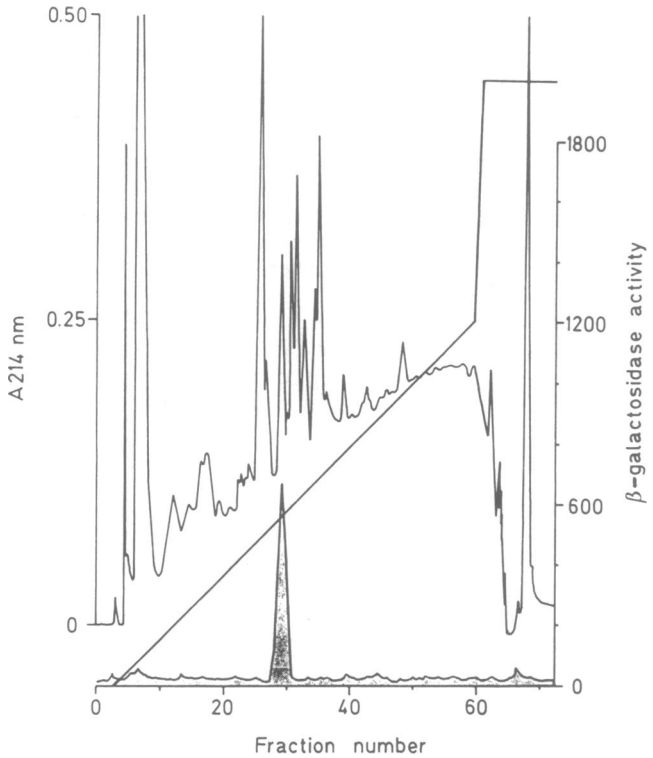

FIG. 1. *T. monococcum* suspension cell exudate contains a single *vir*-inducing activity. Chloroform extract of 27 ml of conditioned medium was fractionated by reversed-phase chromatography/fast protein liquid chromatography (Pharmacia), and each column fraction was assayed for *vir*-inducing activity. The diagonal line represents the elution gradient (0–60% acetonitrile at 1% per min), the solid line gives the UV absorbance of eluted material measured at 214 nm, and the shaded area represents the specific units of β-galactosidase activity induced in the *virB*::*lacZ* tester strain A348-(pSM30).

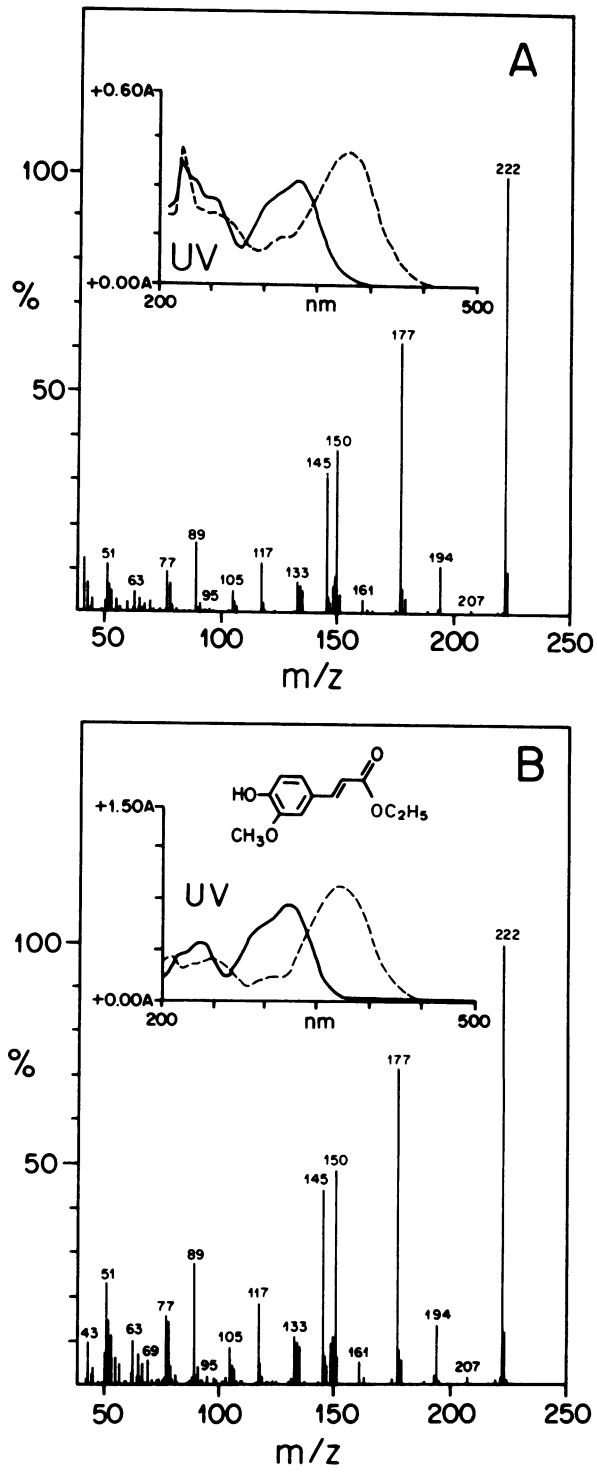

FIG. 2. Identification of *T. monococcum* *vir*-inducing activity as ethyl ferulate by GC/MS and UV spectral analyses. (*A*) GC/MS spectrum of purified *T. monococcum* *vir*-inducing compound. (*B*) GC/MS spectrum of synthetic ethyl ferulate. For each spectrum the relative intensity is plotted against the m/z ratio of the fragment ions. (Insets) Scanning UV spectra, determined in methanol (—) and methanol/sodium methoxide (---).

loss of an ethoxy radical and methanol. Loss of methanol (CH_3OH) is predictive of an ortho configuration between a phenolic hydroxy and methoxy group (21). The ion at m/z 150 is most likely due to the concerted loss of ethylene and carbon dioxide via a McLafferty rearrangement, consistent with ethyl ferulate fragmentation.

Authentic ethyl ferulate was synthesized (see below) and subjected to GC/MS. Fig. 2*B* shows that the resultant spectrum of the synthetic compound is identical to that of the purified wheat molecule. Fig. 2 also shows that the UV absorption spectra of the purified wheat *vir* inducer and synthetic ethyl ferulate are indistinguishable. Both compounds have absorption maxima in methanol at 323 nm, and addition of sodium methoxide results in a 52-nm bathochromic shift with 28% increased intensity. This base-induced red-shift is diagnostic of phenolic compounds, where the phenolic hydroxyl is para to a conjugated ring substituent, such as a ketone or allyl group (5, 22). Further support for the identification of the wheat inducer as ethyl ferulate was given by comparing the GC/MS fragmentation products of purified and synthetic compounds derivatized by trimethylsilylation (data not shown).

Note that the purified wheat *vir* inducer migrated on reverse phase as a doublet coincidental for all biological activity, UV, and mass spectra. The first peak, comprising 22%, eluted at 17.6% acetonitrile, and the second peak, comprising 78%, eluted at 18.1% acetonitrile. These two peaks most likely correspond to *cis*- and *trans*-ethyl ferulate. Although we have not determined the identity of each peak, previous separations of cinnamic acid and its derivatives indicate that cis isomers elute first (23). Hydroxycinnamic acids exist in solution as a mixture of the two isomers, and an equilibrium between the two forms is reached after UV exposure (24); we possibly produced one or the other of the two forms during the extraction procedure.

Preparation of Ethyl Ferulate. To prove the identity of the wheat *vir* inducer required that we assess the chemical and biological properties of authentic ethyl ferulate. As this compound is commercially unavailable, we used the following protocol for its synthesis: an equivalent of ferulic acid dissolved to 20 mM in anhydrous acetonitrile was mixed with four equivalents of absolute ethanol, two and one half equivalents of dicyclohexylcarbodiimide and 0.1 equivalents of F_3CCOOH. After 90 min at 40°C, the reaction was stopped with three volumes of water. Ethyl ferulate partitioned into the aqueous phase and was purified by reversed-phase chromatography. Dicyclohexylcarbodiimide promotes the esterification of primary and secondary alcohols (25); thus, in the reaction with ferulic acid and ethanol, the primary product is ethyl ferulate, and side reactions give (oligo)depsides and -oyldicylohexylureas. Our overall yields were 58.5% ethyl ferulate, 20.8% feruloyldicyclohexylurea [*N*-cyclohexyl, *N*-3-(4-hydroxy-3-methoxy-phenyl)-2-propenoyl, *N'*-cyclohexylurea], 12.5% unidentified hydrophobic despides and ureas, and traces of ferulic acid.

Comparison of Biological Activities of Monocotyledon Versus Dicotyledon *vir* Inducers. To further characterize the ability of monocotyledon cells to induce *vir* activation, we compared the *vir*-inducing activity of the wheat factor relative to the previously identified dicotyledon *vir* inducer, acetosyringone. Concentration/activity curves for acetosyringone and ethyl ferulate were compared (Fig. 3). These data show that the wheat factor, ethyl ferulate, is active at ≈5-fold lower concentration than acetosyringone; i.e., in these experiments, *vir*-inducing activity is first observed between 0.1 and 0.5 μM for ethyl ferulate and at 1 μM for acetosyringone. Interestingly, acetosyringone gives a higher final activity, whereas the half-maximal activities of both compounds are essentially identical.

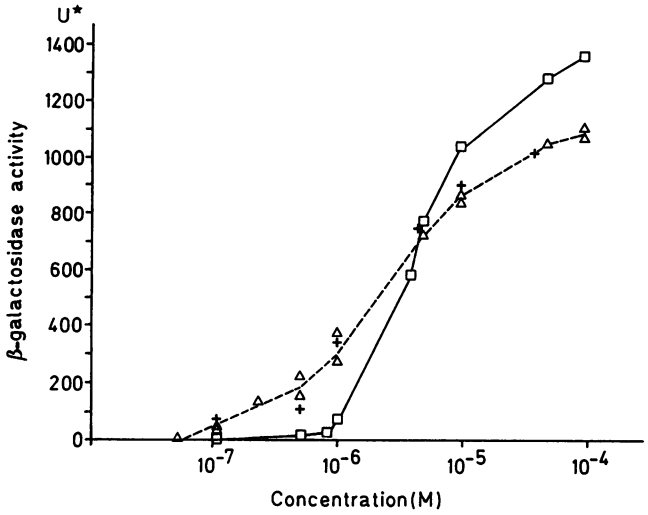

FIG. 3. Comparison of *vir*-inducing activity of ethyl ferulate and acetosyringone. Units of β-galactosidase activity induced in the *virE*::*lacZ* tester strain A348(pSM358) are plotted against concentration of inducer, determined from absorbance measured at its respective λ_{max}. □, Acetosyringone [logε_M, 4.04 at 302 nm; (26)]; +, ethyl ferulate (synthetic; logε_M, 4.23 at 323 nm) (24); △, ethyl ferulate (purified from *T. monococcum*).

Tobacco wound exudate has been demonstrated (5) to contain between 0.5 to 1 μM acetosyringone, sufficient for significant *vir* induction. We calculate that the wheat exudate also contains ≈0.5–1 μM ethyl ferulate (both by monitoring its recovery during purification and by extrapolation of the amount of induction from the original wheat culture exudate with the curve of Fig. 3). Thus, this wheat cell culture produces enough potent *vir*-inducing compound in its exudate to activate the *Agrobacterium* T-DNA transfer process.

DISCUSSION

We have shown that different monocotyledon species produce compounds in their exudates that signal the induction of the *Agrobacterium vir* regulon. Furthermore, we have purified this activity from the spent medium of a *T. monococcum* cell line and identified it as ethyl ferulate. This compound is at least as active at low concentrations as the previously identified dicotyledon *vir* inducer acetosyringone and is produced by the wheat cell line in quantities sufficient for substantial *vir* induction. The molecular properties of ethyl ferulate differ substantially from that of a reported monocotyledon *vir* inducer (15), which was characterized to be large and hydrophilic. Possibly, this large-molecular-weight factor might correspond to ferulic acid esters of monocotyledon cell-wall polysaccharides, as ferulic acid is found esterified to polysaccharides in certain plants, including grasses and corn (27).

Significantly, repeated attempts to transform the wheat cell line with *Agrobacterium* carrying a variety of selectable markers have failed (H. Lörz, personal communication), indicating that some block exists to its transformability, as is generally seen for other monocotyledons. This block is clearly not from a deficiency of *vir*-inducing activity, as *Agrobacterium* can recognize and respond to these cells as targets for transformation. Monocotyledon resistance also is not due to lack of expression of transferred genes because several genes have been successfully expressed in monocotyledon cells after their introduction by chemical and physical methods (11). Thus, a step of the T-DNA transfer process after *vir* activation must be blocked in the *Agrobacterium*–monocotyledon interaction. T-DNA transfer is most proba-

bly mediated via a mechanism analogous to bacterial conjugation (4, 28). An early step of conjugation is the specific binding of donor and recipient cells to establish the stable mating pair. Such cell–cell events probably also are essential to the *Agrobacterium*–plant interaction, and at least two different steps might affect this interaction: an initial nonspecific binding mediated by bacterial exopolysaccharides [synthesized by the *Agrobacterium* chromosomal *chvA* and *chvB* gene products (29)] and a subsequent specific binding (analogous to stable mating pair formation)—probably between *vir*-encoded polypeptides and a particular component of the target plant cell surface. Although *Agrobacterium* binds monocotyledon cells by means of its *chv* gene products (30, 31), it is possible that the *vir*-specified interaction cannot be made with many monocotyledon cells as they lack the necessary ligand for this interaction. Other steps that could be blocked in *Agrobacterium* monocotyledon transformation are the nuclear localization and subsequent integration of the transferred T-DNA intermediate into the cell genome. Perhaps the *Agrobacterium* or plant cell proteins mediating these events in dicotyledons cannot function or are lacking, respectively, in many monocotyledon cells. Conversely, T-DNA integration clearly occurs in the monocotyledon *D. bulbifera* and *A. officinalis*, as shown by DNA hybridization analysis (12, 32). Finally, after integration, the transformed cell must divide and regenerate. However, whereas cell division and lignification usually are stimulated at wound sites in dicotyledons, monocotyledon wound sites typically fail to divide and necrose, as the wounded and adjacent cells rarely proliferate. This fact suggests that monocotyledon cells might be transformable by *Agrobacterium*, but transformants fail to regenerate. Conceivably, monocotyledons might have evolved their necrotic wound response in response to pathogens such as *Agrobacterium*.

Interestingly, the identified dicotyledon and monocotyledon *vir*-inducing molecules are quite similar in structure. In fact, *vir* induction has a very strong molecular specificity for a set of closely related phenolics (ref. 5; S.S., unpublished observations). From all our data we can conclude that a potent inducing phenolic must contain at least one methoxy ortho, and either a conjugated group or hydrogen para to its phenolic hydroxy position. Such structures resemble products of the shikimic acid pathway, which provides plant cells with a broad spectrum of molecules, including the flavonoids and lignins. That *vir* inducers are monocyclic suggests they are more related to lignin precursors and catabolites than to flavonoids. We previously suggested that acetosyringone is produced as part of the lignin repair response in damaged cells. The existence of ethyl ferulate in wheat exudates might reflect a similar process in *T. monococcum*.

We had earlier speculated that the dicotyledon *vir* inducer acetosyringone first attracts *Agrobacterium* to plant wound sites to initiate the T-DNA transfer process (5). Indeed, Shaw *et al.* (9) subsequently demonstrated chemotaxis towards acetosyringone, with a sensitivity threshold below 10^{-8} M (1000-fold below that for maximal *vir* induction). Additional studies have demonstrated that wheat shoot homogenates attract *Agrobacterium* (33). This result concurs with our present finding that wheat cell cultures produce active *vir*-inducing compounds and supports the model that these inducers have two roles, to attract *Agrobacterium* to plant cells that can serve as suitable T-DNA transformation targets and to activate the expression of the *Agrobacterium* functions that mediate T-DNA transfer.

In summary, the resistance of monocotyledon cells to *Agrobacterium* transformation is not from lack of production of *vir*-inducing compounds. Presumably, the defect in this transformation must reside in a step(s) subsequent to *vir* gene activation. This step must be identified before determining whether the *Agrobacterium* system can be modified to affect general monocotyledon transformation. Identification of this step should also lend further insight into the fascinating biology of the T-DNA transfer process.

We are especially grateful to Dr. P. C. Zambryski (University of California, Berkeley) for experimental ideas and invaluable help with the writing of this manuscript. We thank Dr. H. Lörz (Universität Hamburg, Hamburg, F.R.G.) for the wheat, barley, and maize suspension cell cultures and generous advice; Dr. J.-P. Hernalsteens (Vrije Universiteit, Brussels) for the asparagus suspension cell culture; Ir. M. Schelfaut (Rijksuniversiteit Gent) and Dr. M. Claeys (Universitaire Instelling Antwerp, Antwerp, Belgium) for assistance with GC/MS data; and E. T. Wombatt III for encouragement. R.D. is a Research Assistant of the National Fund for Scientific Research (Belgium). This work was accomplished with grants from the Services of the Belgium Prime Minister (O.O.A. 120561.84) to Prof. M. Van Montagu, whose generous support we gratefully acknowledge.

1. Zambryski, P., Tempé, J. & Schell, J. (1989) *Cell* **56**, 193–201.
2. Stachel, S. E. & Nester, E. W. (1986) *EMBO J.* **5**, 1445–1454.
3. Stachel, S. E., Nester, E. W. & Zambryski, P. C. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 379–383.
4. Stachel, S. E., Timmerman, B. & Zambryski, P. C. (1986) *Nature (London)* **322**, 706–712.
5. Stachel, S. E., Messens, E., Van Montagu, M. & Zambryski, P. C. (1985) *Nature (London)* **318**, 624–629.
6. Braun, A. C. (1978) *Biochim. Biophys. Acta* **516**, 167–191.
7. De Cleene, M. & De Ley, J. (1976) *Bot. Rev.* **42**, 389–466.
8. De Cleene, M. (1985) *Phytopathol. Z.* **113**, 81–89.
9. Shaw, C. H., Ashby, A. M., Brown, A., Royal, C., Loake, G. J. & Shaw, C. H. (1988) *Mol. Microbiol.* **2**, 413–417.
10. Engström, P., Zambryski, P., Van Montagu, M. & Stachel, S. (1987) *J. Mol. Biol.* **197**, 635–645.
11. Lichtenstein, C. P. & Fuller, S. L. (1987) in *Genetic Engineering*, ed. Rigby, P. W. J. (Academic, London), Vol. 6, pp. 103–183.
12. Schäfer, W., Görz, A. & Kahl, G. (1987) *Nature (London)* **327**, 529–532.
13. Okker, R. J. H., Spaink, H., Hille, J., van Brussel, T. A. N., Lugtenberg, B. & Schilperoort, R. A. (1984) *Nature (London)* **312**, 364–366.
14. Usami, S., Morikawa, S., Takebe, I. & Machida, Y. (1987) *Mol. Gen. Genet.* **209**, 221–226.
15. Usami, S., Okamoto, S., Takebe, I. & Machida, Y. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 3748–3752.
16. Lörz, H., Baker, B. & Schell, J. (1985) *Mol. Gen. Genet.* **199**, 178–182.
17. Stachel, S., An, G., Flores, C. & Nester, E. W. (1985) *EMBO J.* **4**, 891–898.
18. Hernalsteens, J.-P., Thia-Toong, L., Schell, J. & Van Montagu, M. (1984) *EMBO J.* **3**, 3039–3041.
19. Hooykaas-Van Slogteren, G. M. S., Hooykaas, P. J. J. & Schilperoort, R. A. (1984) *Nature (London)* **311**, 763–764.
20. Kingston, D. G. I., Bursey, J. T. & Bursey, M. M. (1974) *Chem. Rev.* **74**, 215–242.
21. Schwarz, H. (1978) *Top. Curr. Chem.* **73**, 231–263.
22. Scott, A. I. (1964) in *Interpretation of the Ultraviolet Spectra of Natural Products*, eds. Barton, D. H. R. & Doering, W. (Pergamon, Oxford), pp. 89–134.
23. Williams, A. H. (1955) *Chem. Ind. (London)* **74**, 120–121.
24. Comte, P., Zwingelstein, G., Ville, A. & Mentzer, C. (1957) *C. R. Hebd. Séances Acad. Sci.* **245**, 1144–1145.
25. Buzas, A., Egnell, C. & Fréon, P. (1962) *C. R. Hebd. Séances Acad. Sci.* **255**, 945–947.
26. Lemon, H. W. (1947) *J. Am. Chem. Soc.* **69**, 2998–3000.
27. Harris, P. J. & Hartley, R. D. (1976) *Nature (London)* **259**, 508–510.
28. Buchanan-Wollaston, V., Passiatore, J. E. & Cannon, F. (1987) *Nature (London)* **328**, 172–175.
29. Cangelosi, G. A., Hung, L., Puvanesarajah, V., Stacey, G., Ozga, D. A., Leigh, J. A. & Nester, E. W. (1987) *J. Bacteriol.* **169**, 2086–2091.
30. Douglas, C., Halperin, W., Gordon, M. & Nester, E. (1985) *J. Bacteriol.* **161**, 764–766.
31. Graves, A. E., Goldman, S. L., Banks, S. W. & Graves, A. C. F. (1988) *J. Bacteriol.* **170**, 2395–2400.
32. Bytebier, B., Deboeck, F., De Greve, H., Van Montagu, M. & Hernalsteens, J.-P. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5345–5349.
33. Ashby, A. M., Watson, M. D., Loake, G. J. & Shaw, C. H. (1988) *J. Bacteriol.* **170**, 4181–4187.