Glucose inhibition of the characteristic melanoid pigment of *Xanthomonas phaseoli* var. *fuscans*[1]

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The brown melanoid pigment of *Xanthomonas phaseoli* var. *fuscans* is a reliable cultural character to distinguish this pathogen, the cause of fuscous blight of beans (*Phaseolus vulgaris*), from *X. phaseoli*, the causal organism of common bean blight. The pigment appears to be exogenous because it developed mainly in nutrient agar and only slightly in water agar held side by side in the same petri plate inoculated by a single streak of *X. phaseoli* var. *fuscans*. Both pathogens produced similar tyrosinase-type diffusible enzymes in nutrient agar plates as detected by DL-dopa (3,4-dihydroxy-DL-phenylalanine) reaction. Presently unknown specific factors, besides the detectable enzymes, may be involved in pigment production.

High glucose concentration in culture media caused inhibition of pigment development. One of the reasons for this inhibition seemed to be the repressive effect of glucose on the synthesis or secretion of tyrosinase-type enzymes as well as on the autooxidation of substrates, like dopa. Therefore, the amount of glucose in culture media should be carefully regulated when the characteristic brown pigment of *X. phaseoli* var. *fuscans* is used to distinguish this pathogen from *X. phaseoli*.

BASU, P. K. 1974. Glucose inhibition of the characteristic melanoid pigment of *Xanthomonas phaseoli* var. *fuscans*. Can. J. Bot. **52**: 2203–2206.

Le pigment brun mélanoïde de *Xanthomonas phaseoli* var. *fuscans* est un caractère fidèle pour distinguer ce pathogène, la cause de la brûlure foncée de *Phaseolus vulgaris*, de *X. phaseoli*, l'organisme responsable de la brûlure commune chez la fève. Le pigment semble exogène parce qu'il se développe surtout sur gélose nutritive et faiblement seulement sur eau gélosée, ces deux milieux étant placés à côte dans un plat de pétri et inoculés d'une seul trait par *X. phaseoli* var. *fuscans*. Les deux pathogènes ont produit, dans des plaques de gélose nutritive, les mêmes enzymes diffusibles du type de la tyrosinase, telles que détectées par la réaction à la DL-dopa (3-4-dihydroxy-DL-phénylalanine). Des facteurs spécifiques présentement inconnus peuvent être impliqués dans la production des pigments, en plus des enzymes détectables.

Une forte concentration de glucose dans le milieu de culture empêche le développement du pigment. Une des raisons de cette inhibition semble être l'effet répressif du glucose sur la synthèse ou la sécrétion des enzymes du type de la tyrosinase, aussi bien que sur l'auto-oxydation des substrats tel que la dopa. Par conséquent, la quantité de glucose dans le milieu de culture devrait être soigneusement ajustée pour observer le pigment brun caractéristique de *X. phaseoli* var. *fuscans* lorsqu'on veut distinguer ce pathogène de *X. phaseoli*. [Traduit par le journal]

Introduction

Xanthomonas phaseoli var. *fuscans* (Burkh.) Starr & Burkh., the cause of the fuscous blight of beans (*Phaseolus vulgaris* L.), produces a brown melanoid pigment in most bacteriological media (1, 2, 5, 6, 17), by which this pathogen is usually distinguished from *X. phaseoli* (E.F.Sm.) Dows., the causal organism of the common bean blight (5, 6, 17). Burkholder (5) considered the pigment as exogenous and diffusible in nature. He suggested that tyrosinase was probably involved and that glucose might inhibit its production, although his evidence was inconclusive. Dye (6) supported the concept of tyrosinase activity in pigment formation. We have shown that the pigment was similar to melanin and we also observed that tyrosine stimulated but simple sugars retarded its development (1, 2). In many other organisms, it is known that enzymes like tyrosinase are necessary for the production of melanoid pigments (4, 9, 10, 11, 13, 15, 16, 18) and that glucose or its breakdown products may adversely affect some of the enzyme systems or substrates (3, 7, 8, 12, 14, 19). As a taxonomic character, the brown pigment of *X. phaseoli* var. *fuscans* is important (5, 6, 17), but neither the mechanism of its formation nor the process of its inhibition by simple sugars, including glucose, is fully understood. *Xanthomonas phaseoli* does not produce any brown pigment, but it is also not known whether this is because of the lack of the tyrosinase-type enzymes that are believed to

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be produced by *X. phaseoli* var. *fuscans* (5, 6).

To verify the reliability of the brown pigment of *X. phaseoli* var. *fuscans* as a character to distinguish it from *X. phaseoli*, the present work was initiated to determine (1) if the pigment was formed exogenously as a result of tyrosinase activity, (2) if similar enzymes were produced by *X. phaseoli*, and (3) the cause of pigment inhibition by glucose.

Materials and Methods

Cultures of *Xanthomonas phaseoli* var. *fuscans* (Burkh.) Starr & Burkh. and *X. phaseoli* (E.F.Sm.) Dows. were obtained by direct isolation from bean (*Phaseolus vulgaris* L.) seeds and were maintained on Difco nutrient agar at 5°C until required (1, 2, 17).

Pigment Production and Detection of Enzymes

Two assumptions were made to determine if the pigment was exogenously produced in agar media by the catalytic activity of enzymes like tyrosinase: (1) that nutrient agar contained but water agar lacked proper substrates to form melanoid pigment, and (2) that positive dopa (3,4-dihydroxy-DL-phenylalanine) reaction (10, 11) should indicate the presence of tyrosinase-type enzymes in the culture media. Accordingly, culture plates were prepared with both nutrient and water agar in the same plate by allowing one medium to solidify first and then replacing half of it by the other. Each plate was inoculated by a single streak of the test organism placed onto the nutrient agar region adjacent to water agar. After 3–4 days growth at 25°C, agar cups were cut out with a cork borer in both media at various distances (10–40 mm) from the bacterial streak and were filled with a 5000-μg/ml aqueous solution of dopa. Dark brown to black discoloration of the media around the agar cups was indicative of tyrosinase activity by the dopa reaction. Appropriate controls were run with dopa and commercial tyrosinase (Nutritional Biochemical Corporation, Cleveland, Ohio, U.S.A.) without the test organisms.

The Effect of Glucose on Pigment Production

Initially it was speculated that glucose might adversely affect one or more of the processes like synthesis or secretion of tyrosinase, its catalytic activity, or the final conversion of substrates to melanin. Commercial tyrosinase was used for comparative purposes to note the effect of glucose on enzyme activity by dopa reaction in vitro.

The organism was grown in 16-mm test tubes containing 8 ml Dye's medium C (6) in each. Glucose was added to these cultures on the 1st, 7th, 14th, and 21st day after inoculation at four levels of its final concentration (0, 50, 500, and 5000 μg/ml) in eight replicated tubes. The cell-free culture filtrate from each tube was tested for the presence of tyrosinase-type enzymes by adding dopa, also at four levels of its final concentration (0, 5, 50, and 500 μg/ml), every 2nd or 3rd day after glucose incorporation. The final color of the liquid was recorded 48 h after the addition of dopa, and the degree of browning (dark, medium, or light) was expressed as optical density at 405-micron (-μ) wavelength based on light transmittance through Dye's medium C as control. When no browning occurred, a drop (0.06 ml) of commercial tyrosinase solution (100 μg/ml water) was added to determine if the lack of browning was due to the absence of similar enzymes in the culture filtrate.

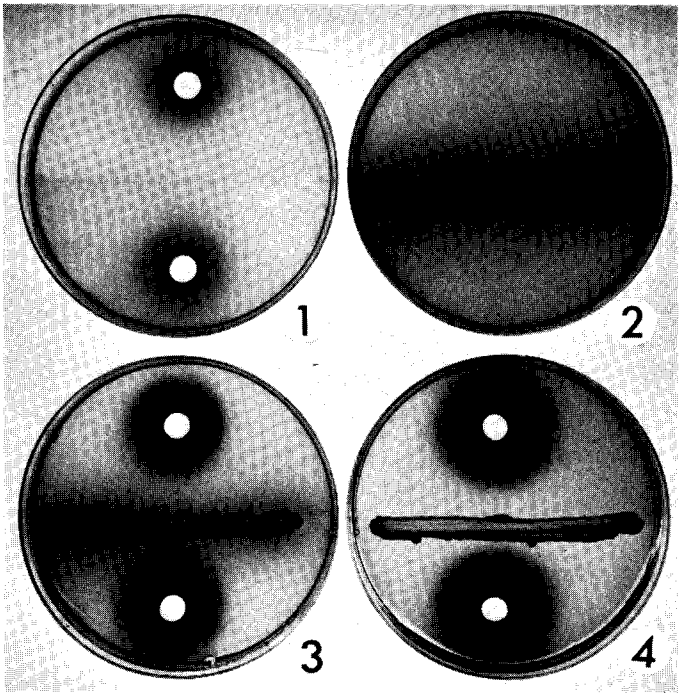
The effect of glucose on the catalytic activity of commercial tyrosinase was noted, in vitro, after mixing various proportions of glucose and dopa solutions (pH 6.0) and adding tyrosinase (0.06 ml of 100-μg/ml aqueous solution) to the mixture at 30°C (10). The production of orange-red color in 3–5 min was indicative of positive dopa reaction (10, 11), while blackish precipitates indicated autooxidation of dopa (10), especially in tubes that were allowed to remain 24 h at room temperature (23 ± 2°C).

The effect of glucose on dopa alone was also checked because this substrate has been regarded as one of the precursors of melanoid pigments (13). Dopa (concentration 2500 μg/ml) in 0.1 M phosphate buffer, Dye's medium C, Difco nutrient broth, and glass-distilled water, with and without glucose, were held at different temperatures (15° to 30°C for 48 h and 100°C for 15 min) to note if glucose would prevent or retard autooxidation of dopa.

Results and Discussion

Pigment Production and Detection of Enzymes

The production of brown pigment by *X. phaseoli* var. *fuscans* and the detection of dopa-sensitive tyrosinase-type enzymes of this pathogen and of *X. phaseoli* on culture plates, each containing half nutrient (lower) and half water (upper) agar, are shown (Figs. 1–4). Dopa alone, placed in agar cups, had no appreciable browning or blackening effect on either nutrient or water agar within the test period (3–4 h), although after several hours (21 h) it produced a blackish stain around the agar cups because of autooxidation (Fig. 1). Commercial tyrosinase caused slight browning of nutrient but not of water agar, indicating that the latter medium lacked suitable substrates to react with the enzyme. *Xanthomonas phaseoli* var. *fuscans* produced distinct brown pigmentation in nutrient agar and only slight brownish discoloration in the adjacent water agar region, after 3–4 days growth (Fig. 2). No discoloration of media was observed on plates inoculated with *X. phaseoli*. After the addition of dopa to similar plates of the two organisms, darkening of media around the agar cups started within the 1st h, indicating positive dopa reaction, and gradually the medium became almost black in 5–6 h (Figs. 3 and 4). This showed that dopa-sensitive tyrosinase-type enzymes originating from both organisms diffused through agar



FIGS. 1–4. Production of brown pigment and detection of tyrosinase-type enzymes of the test organisms by dopa reaction on culture plates, each containing half nutrient (lower) and half water (upper) agar; photographed 21 h after addition of dopa. Fig. 1. Dopa in agar cup as control; blackish discoloration of media due to autooxidation of dopa after several hours. Fig. 2. A single streak of *X. phaseoli* var. *fuscans* producing distinct brown pigmentation on nutrient agar and only slight discoloration of the adjacent water agar. Fig. 3. Darkening of media after the addition of dopa to a similar plate as shown in Fig. 2, indicating presence of enzymes away from the bacterial streak. Fig. 4. Positive dopa reaction on a culture plate of *X. phaseoli*, showing the presence of enzymes but no brown pigment near the streak.

media. The presence of these enzymes away from the bacterial streak and the development of pigment by *X. phaseoli* var. *fuscans* mainly on nutrient agar were indicative of the exogenous nature of the pigment. Slight browning of water agar (Fig. 2) was attributed to the possible diffusion of substrates from the adjacent nutrient agar, which reacted with the enzymes.

Since *X. phaseoli* produced dopa-sensitive enzymes but no pigment, it would appear that specific factors from *X. phaseoli* var. *fuscans*, besides these enzymes, were involved in pigment production. No attempt was made to determine the mechanism of pigment formation and the components or forms (4, 11) of these enzymes as detected by dopa reaction. However, it was clear that the brown pigment was a reliable character for distinguishing the two pathogens because of its specificity.

The Effect of Glucose on Pigment Production

When glucose was incorporated into liquid cultures of *X. phaseoli* var. *fuscans*, greater inhibition of the brown pigment was associated with higher glucose concentration, although the degree of browning increased with increasing amount of dopa (Table 1). After 12 days growth followed by dopa incorporation, the color of cell-free culture fluids exhibited a general pattern of light (L), medium (M), and dark (D) brown, and light yellow (Y). In the yellowish filtrates containing 5000 μg/ml glucose, no change of color was noticed even after the addition of an excess amount of dopa, but an addition of commercial tyrosinase to the mixture showed positive dopa reaction in a few minutes. This indicated that the yellow filtrates did not contain tyrosinase-type enzymes. In vitro, glucose did not affect the catalytic activity of commercial tyrosinase as detected by dopa reaction (Table 2). Therefore, it would appear that the absence of pigment formation in yellow filtrates might be because either the enzymes were not synthesized by the organism or possibly their secretion was affected in the presence of glucose (concentration 5000 μg/ml). A high concentration of glucose (20 000 μg/ml) also prevented autooxidation of dopa as indicated by the lack or delay in the formation of blackish precipitates in phosphate buffer, Dye's medium C, nutrient broth, and glass-distilled water held at different temperatures, from 15° to 30°C, for 48 h. Even after heating for 15 min at 100°C, dopa solution (aqueous) containing glucose remained only slightly straw-colored. Since autooxidation of dopa is one of the final steps in the formation of melanin (13), the prevention of this step by glucose could result in the inhibition of melanoid pigment of *X. phaseoli* var. *fuscans*. It is noteworthy that glucose has been shown to prevent the synthesis of amylase (8) and malic dehydrogenase (19) and to affect the oxidation of β-nitropropionic acid (3).

Results showed that the brown melanoid pigment of *X. phaseoli* var. *fuscans* was a reliable character to distinguish the pathogen from *X. phaseoli*, although both organisms produced similar dopa-sensitive diffusible tyrosinase-type enzymes. The pigment was apparently produced exogenously, confirming Burkholder's idea (5), but presently an unknown specific factor (or factors) of the organism, other than the detectable enzymes, might be involved in its production. One of the reasons for pigment inhibition by glucose seemed to be its repressive effect on the synthesis or secretion of tyrosinase-type enzymes as well as on the autooxidation of sub-

TABLE 1

Browning[a] of 12-day *X. phaseoli* var. *fuscans* culture filtrates (Dye's medium) containing 0, 50, 500, and 5000 μg/ml glucose, 48 h after the addition of 5, 50, and 500 μg/ml dopa (final concentration)

	Glucose concn., μg/ml			
Dopa concn., μg/ml	0	50	500	5000
0	L	L	Y	Y
5	L	L	Y	Y
50	D	M	Y	Y
500	D	D	M	Y

[a]The degrees of browning were light (L), medium (M), and dark (D), with the following ranges of optical densities at 405 μ: 0.119–0.275, 0.658–0.770, and 1.112–1.903, respectively. No browning occurred in yellowish (Y) filtrates (OD range, 0.000–0.096).

TABLE 2

Optical densities[a] of 5 ml glucose and 3 ml dopa mixed aqueous solutions (pH 6.0) at 30°C, 5–8 min after the addition of a drop of 100 μg/ml commercial tyrosinase

	Glucose concn., μg/ml			
Dopa concn., μg/ml	0	500	10,000	20,000
0	0.000	0.001	0.006	0.001
50	0.733	0.699	0.620	0.602
500	0.796	0.710	0.668	0.733

[a]Optical densities, used as a measure of dopa reaction, at 405 μ with 8 ml distilled water plus a drop (0.06 ml) of commercial tyrosinase solution as control. OD of 0.6 or more indicated dark solutions.

strates like dopa. Consequently, the amount of glucose in the culture media should be carefully regulated so that one can observe the production of the melanoid pigment by *X. phaseoli* var. *fuscans* for its taxonomic differentiation from *X. phaseoli*.

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Etude des modes de ramification sympodiale chez *Lycopersicum esculentum* et *L. pimpinellifolium*

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SILVY, A. 1974. Etude des modes de ramification sympodiale chez *Lycopersicum esculentum* et *L. pimpinellifolium*. Can. J. Bot. **52**: 2207–2218.

Chez *Lycopersicum esculentum* Mill. et *L. pimpinellifolium* Dun. les modes de ramification des plantes à ports déterminés et indéterminés sont étudiés par l'observation macroscopique (évolution de l'apex, phyllotaxie et nombre de feuilles des différents rameaux d'une plante adulte) ainsi que par l'analyse des secteurs mutés après traitement de graines. Tandis que la première inflorescence, une cyme, provient de la majeure partie de l'apex préfloral dont le sommet donne la première fleur, les axes de second ordre naissent à l'aisselle des deux dernières feuilles. Chez toutes les variétés, le bourgeon axillaire de l'avant-dernière feuille produit une ramification latérale sympodiale. Par contre celui de la dernière feuille, qui apparaît de façon très anticipée sur le flanc de l'apex préfloral, donne immédiatement une deuxième inflorescence chez les variétés à port déterminé mais constitue l'axe d'ordre deux du sympode principal chez les variétés à port indéterminé. Les caractéristiques de ces deux types de bourgeons sont comparées.

SILVY, A. 1974. Etude des modes de ramification sympodiale chez *Lycopersicum esculentum* et *L. pimpinellifolium*. Can. J. Bot. **52**: 2207–2218.

In *Lycopersicum esculentum* Mill. and *L. pimpinellifolium* Dun. ramification modes of plants with determinate and indeterminate habits are studied by macroscopic observation (apex evolution, phyllotaxis, and leaf number of the different branches of a mature plant) and also by analyzing mutated sectors after seed treatment. While the first inflorescence, a cyme, originates from the greater part of the prefloral apex, the top of which bears the first flower, second-order axes come up at the axils of the last two leaves. In all the varieties, the axillary bud of the second-last leaf produces a sympodial lateral ramification. On the other hand, that of the last leaf, which becomes visible on the side of the prefloral apex, as anticipated, immediately bears a second inflorescence in varieties with determinate habit, but makes up the second-order axis of the main sympodium in varieties with indeterminate habit. The characteristics of these two bud patterns are compared.

Introduction

Les variétés de Tomate présentent un certain polymorphisme (Figs. 1 et 2). Schématiquement une plante adulte paraît constituée d'une tige principale et de rameaux secondaires axillaires. Suivant que l'on a un type à port indéterminé ou déterminé les différentes tiges sont jalonnées d'inflorescences séparées par trois feuilles en moyenne ou portent seulement deux à trois inflorescences. Dans le cas intermédiaire il y a passage progressif du premier type au deuxième par diminution du nombre de feuilles situées entre deux inflorescences.

La formation de la première inflorescence est précédée par un phase végétative. Chez *Lycopersicum esculentum* Mill. on peut classer en plusieurs groupes les différentes théories qui rendent compte de l'initiation florale, selon l'importance de la participation de l'apex. Pour les auteurs les plus anciens la première inflorescence se développait à partir d'un bourgeon axillaire situé immédiatement en dessous de l'apex végétatif (Smith 1935). Cette interprétation n'est plus guère soutenue maintenant et trois processus différents expliquent les transformations de l'apex. Il peut y avoir bifurcation, une partie donnant l'inflorescence et l'autre l'axe de remplacement, soit par "dichotomie" (Venning 1949) soit par un "comportement dissymétrique" (Ecole 1972) comparable à ce qui a été décrit pour le sympode de *Mirabilis jalapa* (Wibaut 1965). Enfin, par un processus inverse du premier, l'apex tout entier peut donner la première inflorescence tandis que l'axe de remplacement provient du bourgeon axillaire situé immédiatement en dessous (Sawhney et Greyson 1972; Brunaud 1973). Il existe également des interprétations intermédiaires. Pour Muller (1940) l'inflorescence est typiquement une prolifération latérale de la tige sans relation avec les feuilles; mais elle peut occasionnellement être terminale

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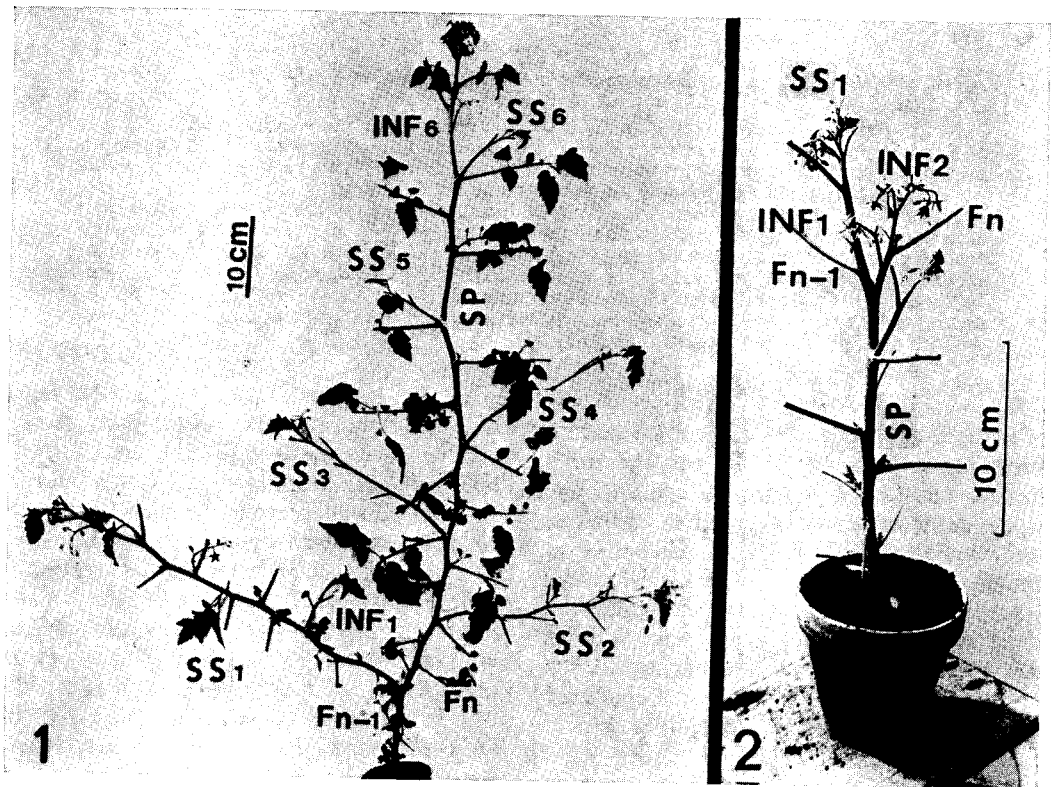
et se développer en même temps qu'une tige végétative. Enfin Wittwer et Aung (1969) font une distinction suivant le port des variétés. Pour les types indéterminés la première inflorescence se forme à partir de l'apex, tandis qu'elle est latérale pour un port déterminé. Toutes ces contradictions se manifestent moins au niveau des documents macroscopiques que des coupes histologiques, une observation incomplète de l'évolution morphologique de l'apex conduisant les auteurs à donner des origines différentes pour le même organe. Actuellement l'interprétation de Sawhney et Greyson (1972), confirmée par Brunaud (1973), paraît la plus vraisemblable.

L'étude que nous avons entreprise a pour but d'une part d'apporter de nouvelles informations sur l'initiation des inflorescences et les modes de ramification, d'autre part de décrire comment ces processus généraux permettent la diversité des ports qui caractérisent les différentes variétés.

Matériel et méthodes

On a employé des variétés de *Lycopersicum esculentum* Mill. à port déterminé (var. Primabel; Fig. 2) ou indéterminé (var. Moneymaker) ainsi que *L. pimpinellifolium* Dun., qui a également un port indéterminé (Fig. 1). Les graines proviennent des sélections de la Société Clause (Brétigny, France) et l'homogénéité des plantes a été vérifiée au cours de plusieurs générations avec autofécondations. Les cultures ont été faites en serres et en pièces climatisées avec les méthodes horticoles classiques.

Trois séries de résultats sont présentées. Tout d'abord les clichés macroscopiques (Fig. 3) ont pour but de donner une représentation complète de l'évolution de



FIGS. 1 et 2. Vue générale. Les fruits et les feuilles sont coupés afin de mettre en évidence la charpente des plantes composée d'un sympode principal (SP) et de sympodes secondaires (SS). F, feuille; INF, inflorescence. Fig. 1. *L. pimpinellifolium*. Fig. 2. *L. esculentum* (Primabel).

FIG. 3. Evolution de l'apex. De gauche à droite: différentes orientations par rapport aux ébauches foliaires. De haut en bas: évolution chronologique vue sous la même orientation. F, *f*, feuille; INF, inflorescence; BAxP, bourgeon axillaire primaire de la dernière feuille; 1 à 6, phase végétative; 7 à 12, phase préflorale; 13 à 27, phase reproductrice; 24, BAxP chez une variété à port déterminé (Primabel); 25, BAxP chez *L. pimpinellifolium*; 27, INF de *L. pimpinellifolium*.