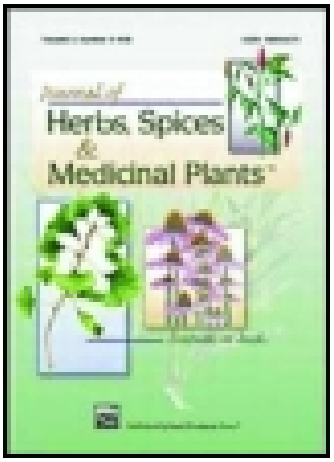

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Breeding for a Hypericum perforatum L. Variety Both Productive and Colletotrichum gloeosporioides (Penz.) Tolerant

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Breeding for a *Hypericum perforatum* L. Variety Both Productive and *Colletotrichum gloeosporioides* (Penz.) Tolerant

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SUMMARY. The acreage of St. John's wort *(Hypericum perforatum* L.), a drug-yielding plant used for its antidepressive properties, considerably increased in Europe over the last few years. In Switzerland, this acreage regularly suffers anthracnose, a disease caused by the *Colletotrichum gloeosporioides* (Penz.) fungus. Our tests were designed to compare 21 wild and 3 commercial varieties on 3 sites with distinct soil climates. This article emphasizes a high genotype variation for this species. We were able to select a genotype that is in agronomical terms more

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satisfactory than the reference variety (Topas). It is dieback tolerant, high-yielding, easy to harvest and should subsequently prove more cost-effective. It blooms early and is thus particularly suitable for growth at high altitude. Finally, its flavonoid and hypericin contents are pharmaceutically promising. It has also been noted that anthracnose is not so virulent at high altitudes and the soil type has an influence on flower production but does not reduce their secondary metabolite contents.

KEYWORDS. St. John's wort, anthracnose, breeding, tolerance

INTRODUCTION

St. John's wort (*Hypericum perforatum*), a member of the Guttiferae family (syn. Hypericaceae, Clusiaceae), has been used for its medical properties throughout the ages (5). It is currently recommended in plant therapy for its antiviral, vulnerary and antidepressive properties (10-13,19,20), and this ability to fight depression is naturally attracting the pharmaceutical industry's interest (11). *H. perforatum* based formulations are used for light or mild depression.

H. perforatum has been the subject of many plant chemistry studies (6,18). However, the flower extract molecules that help fight depression are still unknown. Flavonoids, naphtodianthrons (hypericin and pseudo-hypericin), phloroglucinols (hyperforin) and xanthones are concentrated in flowers and there could be many secondary metabolites to which this medical property might be attributed. This property was for a long time attributed to hypericin (2); that very often remains the analytical reference in the standardization of *H. perforatum* extracts. Recent research work also emphasizes the probable significance of hyperforin (4,8,15).

Antidepressants represent a huge market that provided the impetus for *H. perforatum* development. Although it was still limited a few years ago, the acreage now covers several dozen hectares in France, some hundred hectares in Italy and more than 400 hectares in Germany. A few selected varieties, e.g., Hyperimed, Elixir and Topas, already are commercially available. Topas, a Polish variety registered in 1982, probably is the most extensively available today (14).

In Switzerland, the *H. perforatum* fields were attacked by the *Colletotrichum gloeosporioides* fungus, causing anthracnose starting in 1995 (7). Most of the 20 hectares of *H. perforatum* fields planted in this country are currently managed with a biological specification. Anthracnose can destroy those perennial cultures from the first year, mainly in heavy soil and damp regions (1). Since the specification does not allow the use of fungicides, those cultures usually are irretrievably lost. Hollow lesions circling the stems are noted in infected plants (9,21) (Figure 1); these rapidly turn red as though they were burned, then wither and die (Figure 2).

Médiplant terms of reference. A cooperation protocol was set up in 1996 between Médiplant and Bioforce, a Swiss company in Roggwil/TG, to select a St. John's wort variety that is both productive and *C. gloeosporioides* tolerant. The main selection criterion for this new variety is a *pathogen fungus tolerance*. The *H. perforatum* plots often are in the mountains and an early blooming genotype would thus be quite suitable for growing in altitude. To be quite cost effective, this variety must also be high-yielding in flowery tops and be easy to harvest. Finally, since the antidepressive molecules are not yet known, a plant with a high secondary metabolites content and a chemical profile similar to that of Topas must be sought.

FIGURE 1. *Hypericum perforatum* stem necrosis. Lesions typical of anthracnose caused by *Colletotrichum gloeosporioides*.

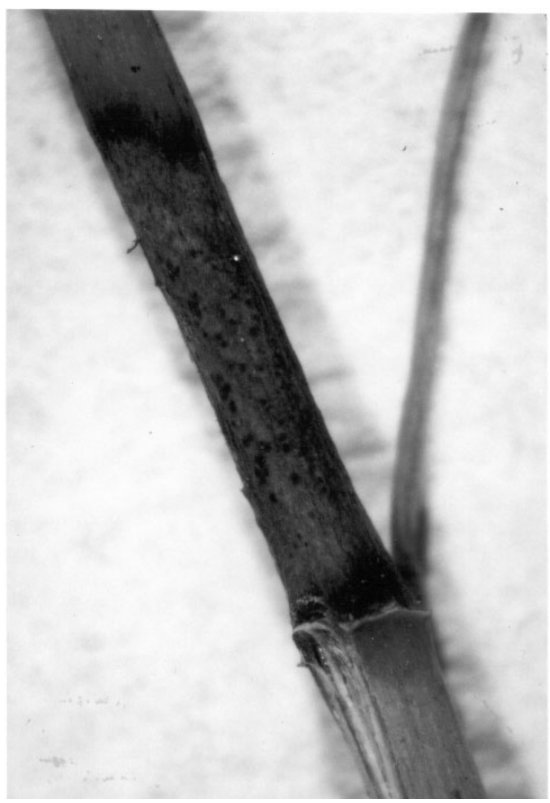
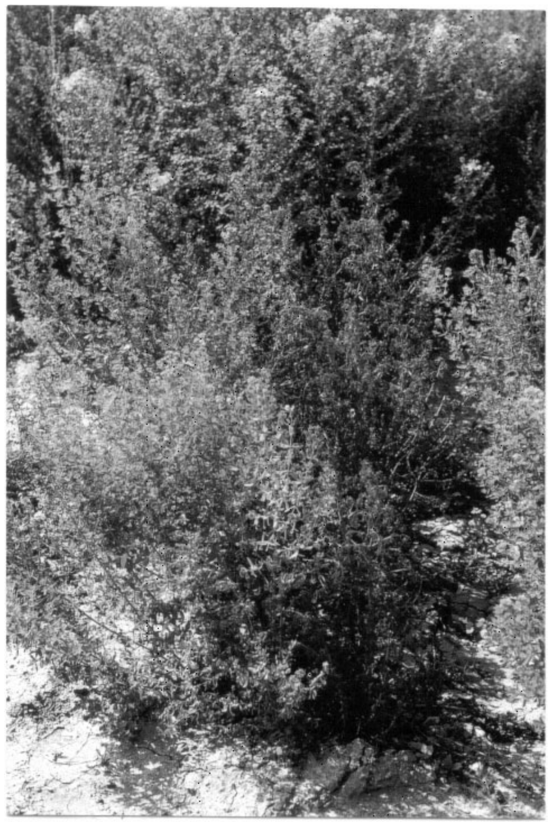


FIGURE 2. *Hypericum perforatum* anthracnose symptoms in the second growth year.



MATERIALS AND METHODS

Seed origin. Twenty-four batches of seed of various origins were compared during a test undertaken in 1997-1998.

Seed origin		Code
3 commercial varieties	Topas	P1
	Hyperimed	P2
	Elixir	P3
21 wild varieties	from Switzerland, Germany, Italy, Australia and Canada	P4 to P24

Topas, the *H. perforatum* variety most extensively available on the market, served as a reference for this test.

Culture sites. Three experimental plots were selected to acquire as much information as possible regarding the behavior of those 24 varieties when cultured.

		Plot 1 Fougères	**Plot 2 Epines**	**Plot 3 Bruson**
Altitude	(in m)	480	480	1060
Soil type	(17)	Silt	Sand	Sandy silt
pH		8	8	7

Experimental design. The experimental design was composed of Fisher blocks with 3 replications in each test station.

Basic plots		**Fougères**	**Epines**	**Bruson**
Number of plants		10	10	10
Surface	(in m^2)	3.2	3.2	2.4
Density	(plants/m^2)	3.1	3.1	4.2

Only 18 accessions out of 24 were cultured in Fougères for lack of available space. Ten plants from the other 6 accessions (P4, P6, P8, P10, P17 and P23) were grown outside the experimental design on the same site.

Culture schedule. The culture schedule is shown below.

Greenhouse seeds	Seed trays	Early March
Greenhouse rooting	Compressed root balls	Early April
Planting	Field	Mid May
Harvesting	Full bloom	June-September

The plants were harvested while they were in full bloom. The stems were cut with shears 15 cm above the inflorescence and folded over by 10 cm after harvest. The experimental surfaces were weeded manually and regularly irrigated as long as the cultures lasted, i.e., 2 years.

Observations and measurements. Plant development and the plots' sanitary conditions were monitored throughout the season. Once harvested, the flowery tops were dried at 35°C for approximately 10 days and weighed. The yields by weight were expressed per plant on a 10-plant per plot basis. Samples were collected and powdered to analyze secondary metabolites (1 analysis per accession and site). Ten flavonoids and two hypericins were quantified by HPLC in the Bioforce laboratory at Roggwil/TG. These measurements were done for every accession collected in 1997. They were repeated in 1998 for some interesting genotypes only.

RESULTS AND DISCUSSION

Out of 21 wild genotypes subjected to tests, P7 was the only one that met the requirements of the 5 initial selection criteria. The results were thus focused on the demonstration of the agronomical qualities of this variety compared to the three commercial ones, mainly Topas.

Anthracnose tolerance. Figure 3 reports the sanitary conditions of the *H. perforatum* plants tested after two years' culture. Anthracnose is quite virulent in the plains–94% of the plants growing on the Epines plot and 89% of those growing on the Fougères plot were dead or diseased after the second test year. The commercial varieties' rate of attack was 64 to 100%; it was only 17% (Fougères) and 50% (Epines) for genotype P7. An analysis of variance (ANOVA) followed by a Newman-Keuls test ($\alpha = 5\%$) indicated that genotype P7 was, on the Fougères plot, significantly more dieback tolerant than the *H. perforatum* varieties currently available on the market. On the Epines site it was statistically comparable to Topas, the reference variety, as well as genotype P17. These three plant types were more dieback resistant than the other *Hypericum* accessions grown in this experimental device.

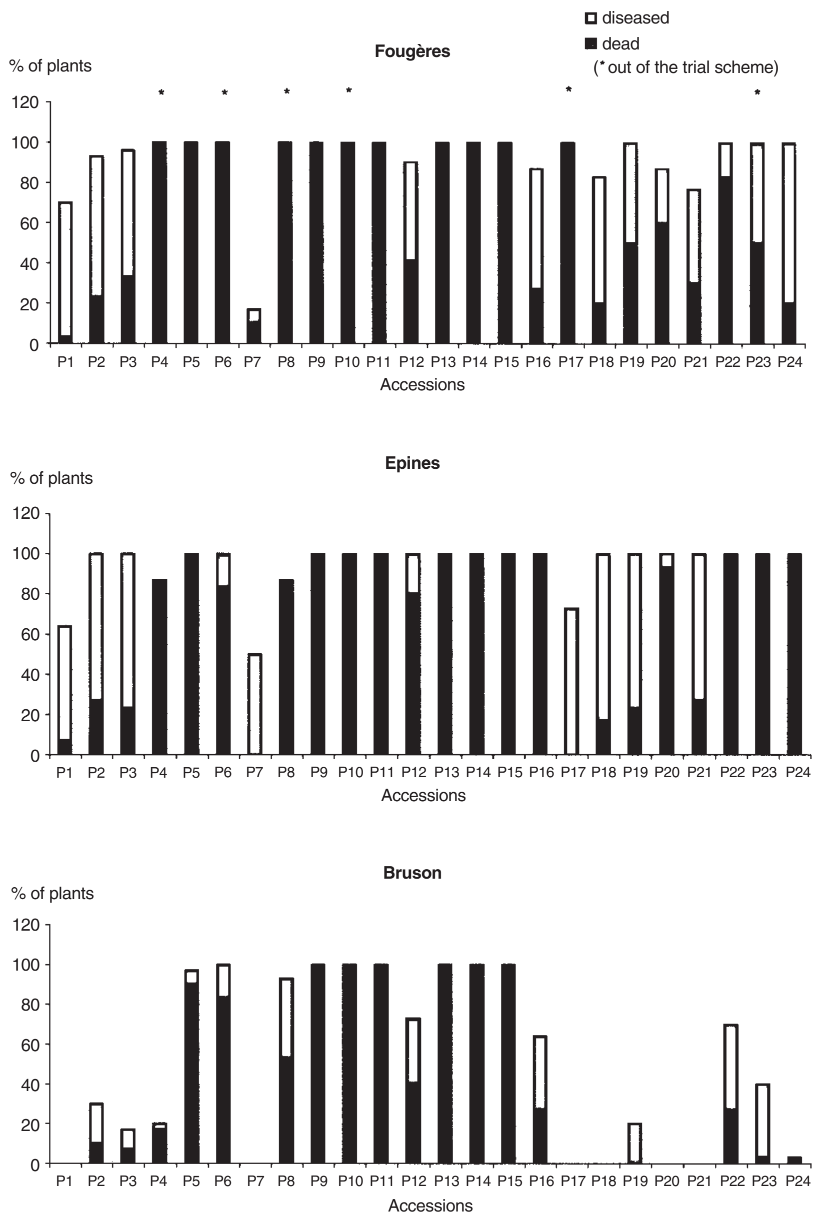
Anthracnose did not seem so virulent on the Bruson site in high altitude. During the 1998 harvest, 49% of the plants were still healthy. Furthermore, 6 of the 24 genotypes were totally symptom free, the Topas and P7 varieties amongst them; 30% of the Hypermed plants and 17% of the Elixir ones were diseased.

Phenotypes. Highly different growing modes were noted between batches throughout the first year. The accessions differ as to how they hold themselves up, how their main stems are branched and how homogenous their flower horizon is. These are classified into five phenotype categories:

1. Erect plant, no base branching, compact flower horizon
2. Erect plant, little base branching, compact flower horizon
3. Irregular plant, heavy base branching, large flower horizon
4. Irregular plant, heavy base branching, vague flower horizon
5. Creeper

We did not observe any significant morphological variations between plants of a same variety. The specific mode of sexual reproduction of this spe-

FIGURE 3. Dieback level in the second year for 24 *Hypericum perforatum* accessions on three growth sites (notes made upon harvest in 1998).



cies, apomixis, is probably responsible for this feature (3,16). P7 belongs to the first of the five phenotype categories. It is easy to harvest, thanks to its erect stand, highly homogenous flower horizon and flowers laid out in a same plane. Categories 3, 4 and 5 are more difficult to harvest because the corymbs are at different heights. The commercial varieties, Topas and Hyperimed, are in the third category. Elixir belongs to the fourth one. The morphological differences between plants were reduced during the second year. Every plant had an erect stand and a varying number of vertical stems. P7's advantage over Topas was a very compact flower horizon (Figure 4).

Blooming. In the first year, *H. perforatum* was harvested in the plains from July 8 to August 20. The next year, the plants bloomed one month earlier and were harvested from June 9 to July 13. In the mountains, the harvest was one month late and the difference between the first year (August 13-September 4) and the second one (July 6-August 4) remained. Early, intermediate or late genotypes were determined according to the harvest dates (Figure 5). The Topas variety probably had, amongst all those considered, the longest growing period before blooming. In the mountains, this late genotype, as 6 others, did not bloom early enough to be harvested during the first year; it was the last to be harvested in Bruson in 1998. P7, on the other hand, is in the early blooming category. In 1997 in the plains, it was blooming approximately one month be-

FIGURE 4. Selected genotype (P7) blooming during the second year. Its erect stand and homogenous flower horizon is to be noted.

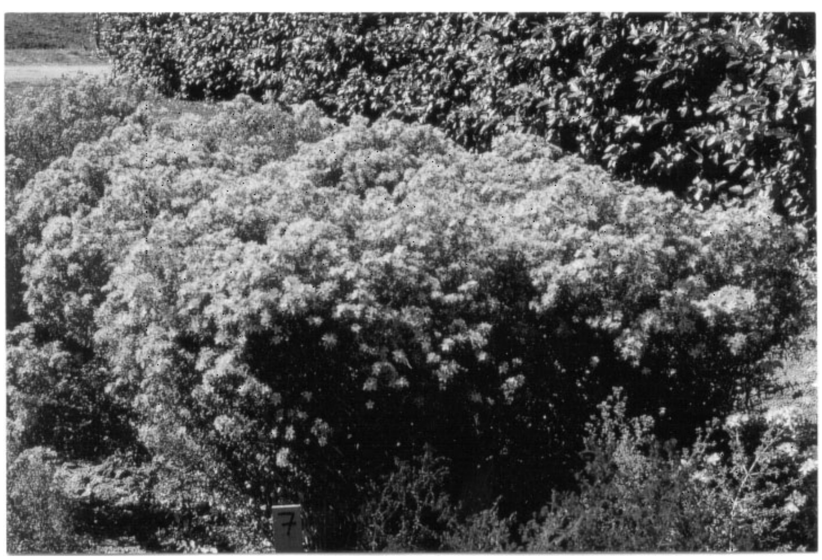
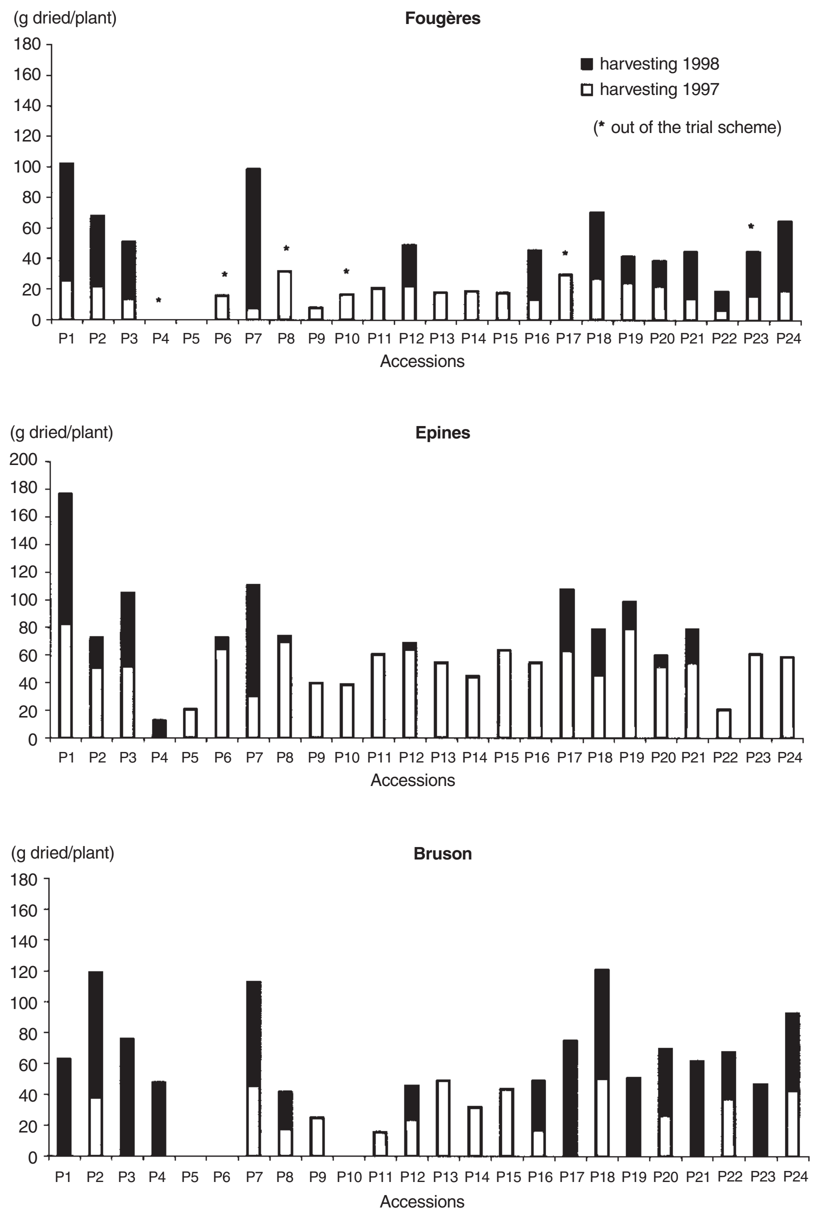


FIGURE 5. Early blooming differences between genotypes P7 (on the left) and Topas (on the right).

fore Topas, the reference variety. In 1998, it was blooming two weeks before Topas. It can thus bloom at high altitude from the first year. Hyperimed also blooms early. Elixir blooms intermediate to late and could not be harvested in Bruson in 1997.

Yields by weight. Their dieback sensitivity excepted, the soil type, the plot's altitude and the morphology of the plants also have an influence on the flower yield of the *H. perforatum* accessions (Figure 6). The results demonstrated that flowery tops production is improved on a properly irrigated sand rather than silt soil. The plants developed three times more flowers the first year on the Epines site compared to the Fougères one. Epines' sandy soil is particularly suited for Topas and its cumulative yield over two years (176 g) could not be exceeded by the other accessions tested on this site (21 to 111 g). Stems without base branches and very early blooming proved detrimental to genotype P7 during the first year in the plain sites. Its bloom production was 1.5 to 3 times lower than that of the three commercial varieties. However, in the second year, this yield (31 g in Fougères and 80 g in Epines) was comparable to that of Topas, the best of the three commercial varieties (76 g in Fougères and 93 g in Epines). This early blooming gives P7 an incomparable advantage in high altitude. In Bruson, its cumulative yield over two years (113 g) exceeded that of Topas (63 g) and Elixir (76 g) that did not bloom until the second season. It

FIGURE 6. Flowery top yields for 24 *Hypericum perforatum* accessions on three sites over two years.



provided roughly the same quantity as Hyperimed (119 g). Even if the cumulative yields of P7 in the plains were not significantly higher than those of Topas, this genotype was still one of the most productive plants analyzed during those tests (ANOVA; Newman-Keuls test, $\alpha = 5\%$).

Dosed substances contents. The flavonoid and hypericin (hypericin and pseudo-hypericin) contents quantified in 1997 for the Epines plants are presented in Table 1. Amongst flavonoids are included rutin, hyperoside, isoquercitrin, quercitrin, quercetin and biapigenin; four of these remain unidentified. Globally, a very interesting variation was evidenced for the substances dosed as a whole, with, for example, extreme values ranging from 1 to 7.5 for hypericin. It must be noted that Topas apparently had the least flavonoids and hypericins. Genotype P7 appears promising for the preparation of pharmaceutical extracts; it includes the same range of measured substances as Topas and also contains 26% more flavonoids and 79% more hypericins compared to the latter.

The chemical profile defining the quality of the *H. perforatum* flowery tops is dependent upon the plant development stage upon harvest (14) but it does not seem to be influenced by the soil type, the altitude or the culture's age (Figure 7). This principal component analysis demonstrated, for each of the four accessions analyzed, a very low scatter of the chemical profiles whatever the site or the harvesting year may have been.

CONCLUSION

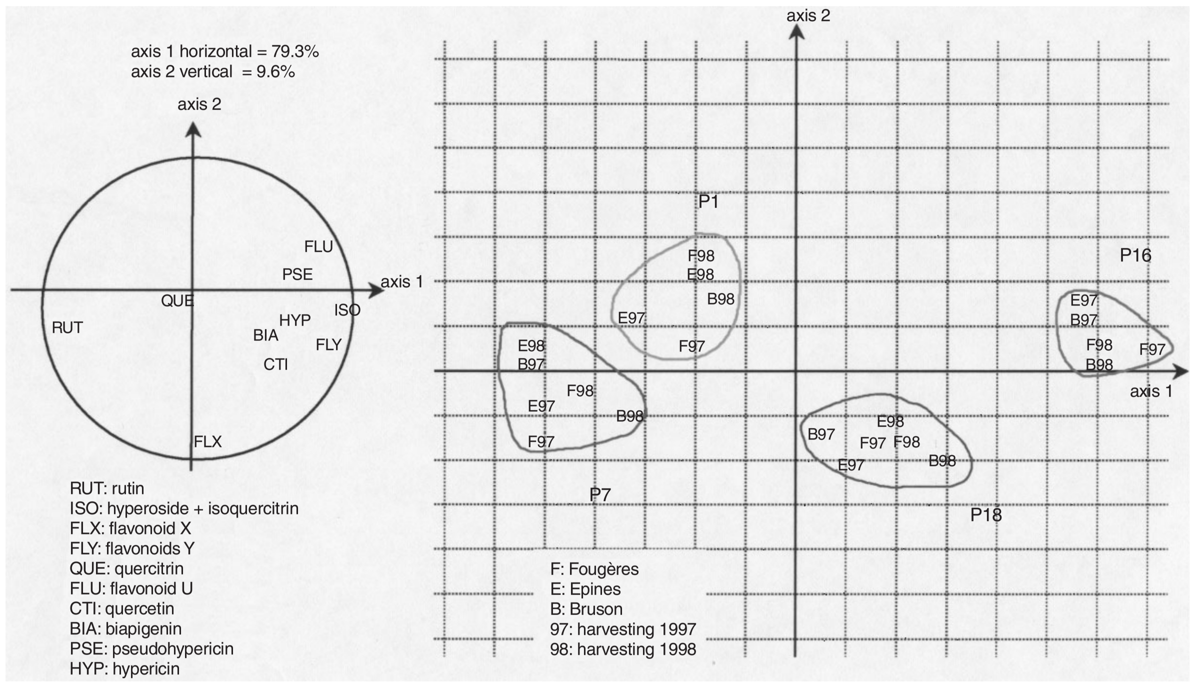
The high variation detected for the five selection criteria initially retained provided interesting vegetable material and allowed for satisfactory selection.

TABLE 1. Flavonoid and hypericin contents (in mg/100 g of dried flowery tops) for 24 *Hypericum perforatum* accessions harvested in full bloom during the first year (Epines site, 1997).

Accessions	rutin	hyperoside + isoquercitrin	flavonoid X	flavonoids Y[1]	quercitrin	flavonoid U	quercetin	biapigenin	pseudohypericin	hypericin	**Total Sum of Flavonoids** content	*index*	**Total Sum of Hypericins** content	*index*
P1 (Topas)	910	1343	104	39	97	0	119	82	125	34	2693	*100*	159	*100*
P2 (Hyperimed)	411	1209	380	408	227	0	169	322	236	108	3125	*116*	343	*216*
P3 (Elixir)	500	1559	482	408	189	56	200	256	128	58	3649	*135*	185	*116*
P7	1024	1016	453	176	411	0	93	213	204	80	3384	*126*	284	*179*
Mean	**711**	**1282**	**355**	**258**	**231**	**14**	**145**	**218**	**173**	**70**	**3213**	**-**	**243**	**-**

(1) 2 peaks

FIGURE 7. Principal component analysis of the chemical composition of the dried flowery tops of four *Hypericum perforatum* varieties grown on three sites over two years.



A *H. perforatum* variety tolerating the severe anthracnose problem and well suited for mountain growth was selected. The flowery top yield of this new genotype is competitive compared to that of the *H. perforatum* varieties currently available on the market. In addition, its secondary metabolite chemical profile meets industrial requirements (Table 2). This variety is now being registered. Tests are currently in progress to optimize its growth.

TABLE 2. Comparing three commercial *Hypericum perforatum* varieties with the genotype selected.

Selection criteria		Topas	Hyperimed	Elixir	P7
1. anthracnose tolerance		average	low	low	**good**
2. blooming	1st year	late	intermediate	late	**early**
	2nd year	late	intermediate	intermediate	intermediate
3. flower horizon		large	large	vague	**compact**
4. flower yield		**good to very good**	**good**	average	**good to very good**
5. contents	flavonoids	low	low	**high**	**good**
	hypericins	low	**high**	low	**good**
Remarks			No longer sold	Heterogenous, does not appear to be a true variety	

Boldface indicates advantageous traits.

REFERENCES

1. Bomme U. 1997. Produktionstechnologie von Johanniskraut (*Hypericum perforatum* L.) (German) (Production technology of St. John's-Wort (*Hypericum perforatum* L.)). *Z. Arzn. Gew.pfl.* 2:127-134.

2. Bruneton J. 1993. *Pharmacognosie–Phytochimie des plantes médicinales* (French) (Pharmacognosy–Phytochemistry of Medicinal Herbs). Lavoisier TecDoc, Londre, Paris et New York. 915 pp.

3. Cellarova E., R. Brutovska, Z. Daxnerova, K. Brunakova, and R.C. Weigel. 1997. Correlation between hypericin content and the ploidy of somaclones of *Hypericum perforatum* L. *Acta Biotechnol*. 1:83-90.

4. Chatterjee S.S., M. Nöldner, E. Koch, and C. Erdelmeier. 1998. Antidepressant activity of *Hypericum perforatum* and hyperforin: the neglected possibility. *Pharmacopsychiat*. 31(special issue):7-15.

5. Czygan F. 1993. Kulturgeschichte und Mystik des Johanniskrautes (German) (History and Mysticism of St. John's-Wort). *Zeitsch. Phytoth.* 14:272-278.

6. Debrunner N., and X. Simonnet. 1998. Le millepertuis: une plante médicinale antidépressive d'un très grand intérêt (French) (St.-John's-wort: an antidepressive medicinal plant of interest). *Revue Suisse Vitic. Arboric. Horic*. 30(4):271-273.

7. Debrunner N., A.L. Rauber, A. Schwarz, and V. Michel. 2000. First report of St. John's-Wort anthracnose caused by *Colletotrichum gloeosporioides* in Switzerland. *Plant Dis*. 84(2):203.

8. Erdelmeier C.A.J. 1998. Hyperforin, possibly the major non-nitrogenous secondary metabolite of *Hypericum perforatum* L. *Pharmacopsychiat*. 31(special issue): 1-6.

9. Hildebrand P.D., and K.I.N. Jensen. 1991. Potential for the biological control of St. John's-Wort (*Hypericum perforatum*) with endemic strain of *Colletotrichum gloeosporioides*. *Can. J. Plant Path*. 13:60-70.

10. Hobbs C. 1989. St. John's Wort (*Hypericum perforatum* L.)–A review. *Herbal Gram* 18/19:24-33.

11. Hölzl J. 1993. Inhaltstoffe und Wirkmechanismen des Johanniskrautes (German) (Content and Efficiency of St. John's-Wort). *Zeit. Phytother*. 14(5):255-264.

12. Hostettmann K. 1997. *Tout savoir sur le pouvoir des plantes sources de médicaments* (French) (All about the Power of Plants Sources of Medicines). Favre, Lausanne. 239 pp.

13. Hostettmann K., O. Potterat, and J.L. Wolfender 1994. Millepertuis et gentianes: nouveaux antidépresseurs? (French) (St.-John's-Wort and Gentians: New Antidepressants?). *In Ressources et potentiels de la flore médicinale des Alpes*. actes du troisième colloque Médiplant, 20 Octobre, 1994, Bruson, Suisse. pp. 51-58.

14. Kartnig T., B. Heydel, L. Lässer, and N. Debrunner. 1997. Johanniskraut aus schweizer Arzneipflanzenkultur (German) (St.-John's-Wort cultivated as a medicinal herb in Switzerland). *AgraForsch*. 4(7):299-302.

15. Laakmann G., C. Schüle, T. Baghai, and M. Kieser. 1998. St. John's-Wort in mild to moderate depression: the relevance of hyperforin for the clinical efficacy. *Pharmacopsychiat*. 31(special issue):54-59.

16. Martonfi P., R. Brutovska, and E. Cellarova. 1996. Apomixis and hybridity in *Hypericum perforatum. Folia Geobot. Phytotax.* 31(3):37-44.

17. Rod P. 1980. Granulométrie–Nouvelles limites, nouvelles définitions (French) (Granulation–New limits, new definitions). *Revue suisse Vitic. Arbor. Hortic*. 12(2): 1-8.

18. Roth L. 1990. *Hypericum–Hypericin* (German). Ecomed, Landsberg. 158 pp.

19. Schaffner W., B. Häfelfinger, and B. Ernst. 1992. *Compendium de phytothérapie* (French) (Compendium of Phytotherapy). Arboris-Verlag, Hinterkappelen, Bern. 336 pp.

20. Schauenberg P., and F. Paris. 1977. Delachaux et Niestlé, eds. *Guide des plantes médicinales* (French) (Medicinal and Aromatic Plants Guide). Neuchâtel. 386 pp.

21. Schwarczinger I., and L. Vajna. 1998. First report of St. John's-Wort anthracnose caused by *Colletotrichum gloeosporioides* in Hungary. *Plant Dis*. 82:711.