# Germination tests of four Rubus species under immediate sowing after harvest

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# Naohiro Naruhashi[1], Keisuke Ban[1,2] and Tomoko Yamada[1,3] : Germination tests of four *Rubus* species under immediate sowing after harvest

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The genus *Rubus* consists of more than 250 species and is widely distributed in the world (Airy Shaw 1973), from tropical to subarctic regions, showing great variations of their habitats. In Japan, *Rubus* can be found from subtropical regions to cool temperate or alpine ones of mountains (Naruhashi and Satomi 1972, 1973).

Although Jennings (1988) summarized the germination characteristics of the genus *Rubus*, yet data for most of the wild species is still lacking. Naruhashi et al. (1999) examined the germination pattern of 47 indigenous and cultivated species of *Rubus* in Japan, and classified them into the following four germination types under immediate sowing after harvest: 1) high geminations group (10 species), 2) middle germination (7 species), 3) low germination (7 species), and 4) no germination (23 species). Furthermore, the authors found that the germination rates of *Rubus* inhabiting the temperate regions showed wider inter- and intra-specific variations as compared to those in other climatic regions.

In the experiments by Naruhashi et al. (1999), three temperate spceies showed remarkable intraspecific variations of the germination rate, depending on localities or populations: *Rubus crataegifolius* (0-27%), *R. hirsutus* (0-49%), and *R. palmatus* (0-49%). However, these results were obtained from a small number of samples (localities), therefore it is necessary to examine the germination traits from a high number of localities in order to confirm the intraspecific variations in those species. *Rubus parvifolius* showed 0-3% in germination rate (Naruhashi et al. 1999), but is distributed throughout Japan. In the present study, we selected four *Rubus* species as mentioned above, and reinvestigated intraspecific variations of germination rate, in relation to environmental gradients; i.e. latitude and altitude of collected samples.

## Materials and methods

We used the seed samples of thirty different localities of *R. hirsutus* Thunb. and *R. crataegifolius* Bunge and carried out germination tests in 1998. Fifty two samples of *R. palmatus* Thunb. and forty three samples of *R. parvifolius* L. were used in the tests conducted in 1999. In this paper, the word, "seed" is botanically equivalent to pyrenes.

Fruits consisting of aggregate fruitlets were peeled with an electric mixer without damage to the surface of seeds on the harvest day or within a few days after harvest. The relevant data of the samples of each species are cited in the Appendix.

The seeds sterilized for 20 min using 2% sodium hypochlorite solution, were transferred to sterilized 1% agar media with distiled water in a test tube. Operations were done on a clean bench and seeds were kept at alternating temperatures of 20℃ for 12 hr following 30℃ for 12 hr under continuous light in an incubator. 100 seeds for each sample were sown and germination rate was scored after 100 days.

## Results and discussion

Germination rates of samples of the four species showed infraspecific variation. The rate in *R. crataegifolius* varied from 0 to 6% (Appendix, Fig. 1), *R. hirsutus* from 0 to 38% (Appendix, Fig. 1), *R. palmatus* from 1 to 89% (Appendix, Fig. 1),

and *R. parvifolius* from 0 to 9% (Appendix, Fig. 1). In *R. crataegifolius* in the previous report (Naruhashi et al. 1999) a rate of 27% was shown to be an exceptional result. However, excepting that performance both results pertaining this species were practically alike - namely the seeds of the species hardly germinated. In *R. hirsutus* most samples showed lower than 10% of germination rate, and the maximum rate was 38%. This species too showed almost the same result, i.e., hardly germinated and rarely better germinated. In the previous report *R. palmatus*

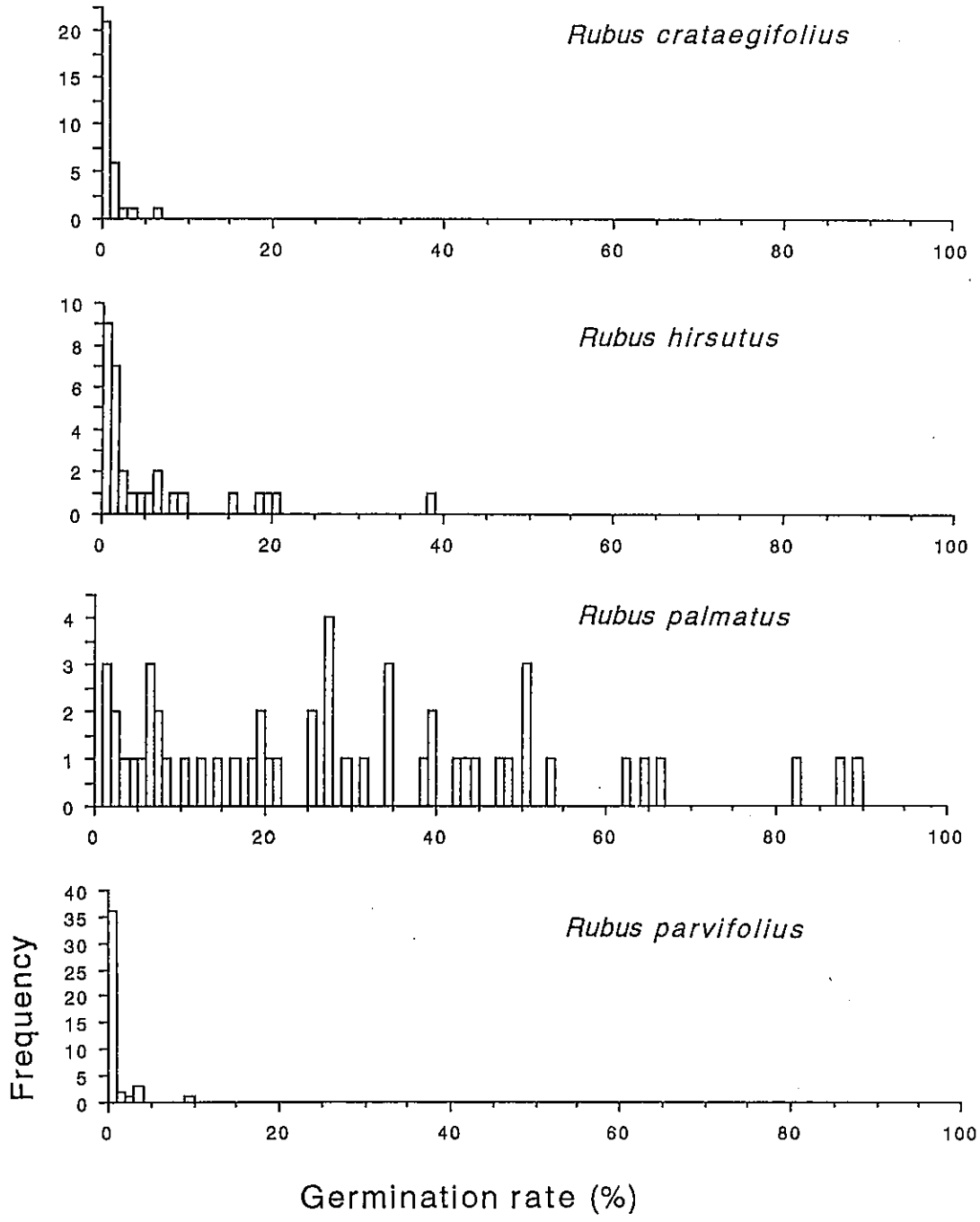


Fig. 1. Germination rate and their frequencies in four *Rubus* species.

showed the remarkable variation ranging from 0 to 49% with a mean of 14.5 %, and in the present examination the variation of the germination rate was also from 0 to 89% with a mean of 30.2%. This time the germination rate of the species is higher than before. However, roughly speaking in both examinations also this species showed the same result. Namely this species showed the whole range of germination types, i. e., best germinated, better germinated, hardly germinated and not germinated as reported in previous paper (Naruhashi et al. 1999). In *R. parvifolius* only one datum of collection showed 9%, however the others were not germinated, ranging from 0 to 3% with a mean of 0.5%. This species also showed almost the same result as seen in the previous report.

The lower rate of germination or no germination at all of seeds in *R. crataegifolius* and *R. parvifolius* can be interpreted in terms of the dormancy of seeds. Seeds produced by species distributed in areas with distinct seasons can germinate in spring after dormancy, this matter being an adaptive phenomenon to the environment. Namely the period of fruit maturity in the species is mainly June to July in *R. crataegifolius* and mainly July to early August in *R. parvifolius*. If the seeds of these species germinated after one month, juvenile and non hardy plants may meet severe weather both in autumn as well as after winter season before enough growth has taken place. In fact, most seeds of these species do not at all germinate as mentioned above. These seeds may be met with in the winter coldness. From the result of the moist-chilling experiment (4℃, 100 days; Naruhashi et al. unpublished), the germination rate was not higher. This may mean that the seeds need lower temperatures and longer period of winter coldness.

After a forest fire we know that many plants germinate as pioneer plants. However, Cong and Kikuchi (1998) reported no germination resulting from heat pretreatment tests (5 min at 50 −150 ℃) of *R. crataegifolius* and *R. parvifolius*, and *R. palmatus* as well.

The germination rate of seeds in each locality of *R. hirsutus* is shown in the Appendix. As compared with the two species above it showed better germination (Fig. 1). The habitat of the species is open places, edge of forests and rarely under evergreen forests (Naruhashi and Satomi 1972, 1973) and the maturity time of its fruits is in May. Therefore, some seeds may germinate in summer and others may germinate next year. This agrees with the results of the moist-chilling treatment (4℃, 100 days), being in some cases effective and in others not effective for germination, depending on the samples used (Naruhashi et al. unpublished).

The germination rate of seeds in each locality of *R. palmatus* is shown in the Appendix. The samples were collected from lower latitude of Kagoshima Pref. to higher latitude of Akita Pref. Sperman rank correlations between germination rate and latitude are shown in Table 1. Statistically no correlation between these two parameters could be detected at the $p>0.1$ significance level. This fact was the same in the germination of the other three species. In Sweden Nybom (1980) reported that the seed of blackberies (*Rubus* subgen. *Rubus*) from northerly populations and from zones with severe climate tend to germinate earliest. However, in the present experiment we have never observed any correlation between earliness of germination and lati-

Table 1. Sperman rank correlations (rs) between germination rate (%) and altitude, or latitude of collection, in four *Rubus* species

| | *R. crataegifolius* germination rate (n = 30) | *R. hirsutus* germination rate (n = 30) | *R. palmatus* germination rate (n = 52) | *R. parvifolius* germination rate (n = 43) |
|---|---|---|---|---|
| Latitude | 0.015<br>p = 0.9366 | −0.086<br>p = 0.6420 | 0.145<br>p = 0.3488 | 0.145<br>p = 0.3488 |
| Altitude | −0.100<br>p = 0.5895 | −0.145<br>p = 0.4335 | −0.079<br>p = 0.6099 | −0.079<br>p = 0.6099 |

tude, i.e., climate.

Also no correlation exists between germination rate and altitude. Moreover, the reasons why high percentage of germination rate among samples in each species occurred, are not explained.

The seed size of four *Rubus* species ascertained as mean of 30 seeds in a locality is as follows: *R. crataegifolus* (1.81mm in length, 1.01 mm in height, 0.81mm in width), *R. hirsutus* (1.42, 0.82, 0.61), *R. palmatus* (1.98, 1.20, 0.86) and *R. parvifolius* (2.15, 1.24, 0.95). Generally speaking, seeds of small size have no or weak dormancy, and seeds of large size have strong dormancy. In the present four species, however, no relationship between germination rate and seed size was evident.

The maturity time of the fruits is mainly in June. Some seeds of these species may germinate in summer and others may germinate next year. In the species moist-chilling treatment is very effective, i.e., 47.6% higher than control as mean value in 1995 and 33.2% higher than control in 1997 (Naruhashi et al. unpublished).

The seeds of *Rubus* are generally known to be dispersed by birds and mammals (Ridley 1930). After dispersal, some seeds can be incorporated into soil seed bank (Toyooka and Sugawara 1980; Nakagoshi 1984 a, 1984 b, 1985, 1992; Hirabuki 1988). Suzuki (1997) reported that after 8 or 9 months from burial, the germination rate of seeds markedly increased in two Japanese *Rubus*, *R. palmatus* and *R. parvifolius*.

Disseminules of *Rubus* are at first fruitlets consisting of aggregate fruit, but they are secondary seeds consisting of endocarp and real seed after digestion of outer fruit-coat by birds and mammals, or after getting rotten by fungi and bacteria. Even though seeds are stored for long time under soil or on the soil surface with litter cover, they can germinate when their places are disturbed due to environmental changes such as receiving light and diurnal temperature fluctuation. These traits of the dispersal and germination pattern are common characteristics of *Rubus*, and explain why the germination rate of the seeds is very low under immediate sowing after harvest.

In closing, *Rubus* is thought to be adapted to two different types of dispersal of disseminules, i.e. spatially by birds and mammals, and in relation to time as soil seed bank by means of strong dormancy. In addition to this property, *Rubus* seeds act as mass in diet and droppings of animals for small aggregate fruitlets, to have no constant germination rate, i.e. containing one or two exceptional seeds for germination, and variation among populations is accounted for adaptation in terms of the start of colonization to new niches.

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**鳴橋直弘[1]・番 敬亮[1,2]・山田智子[1,3]：採り蒔きによるキイチゴ属4種の「種子」の発芽**

鳴橋他（1999）は日本産と栽培の47種のキイチゴ属の種子（正確には小核）の採り蒔き実験をし，よく発芽するもの，かなり発芽するもの，少し発芽するもの，発芽しないものにタイプ化し，報告した。今回は産地によって差のある4種をとりあげ，それらの変異について調べた。

クサイチゴ（*Rubus hirsutus* Thunb.）30カ所，クマイチゴ（*R. crataegifolius* Bunge）30カ所，モミジイチゴ（*R. palmatus* Thunb.）52カ所，ナワシロイチゴ（*R. parvifolius* L.）43カ所から集めた種子の発芽実験をおこなった。実験方法は，種子は採り蒔きを原則とし，ミキサーで果肉を取り，100粒を試験官の寒天培地に蒔き，インキュベーターで光照射下，12時間20℃と12時間30℃のくり返しで行い，100日間の結果を発芽率とした。

材料の種子の採集場所，標高，採集日，採集者，及び発芽率はAppendixとFig. 1に示した。クマイチゴ（発芽率0～6%，平均0.6%）とナワシロイチゴ（発芽率0～9%，平均0.4%）はほとんど発芽しなかった。クサイチゴは0～38%であり，やや発芽するものから発芽しないものまであり，平均5.4%であった。一方，モミジイチゴは0～89%で，平均14.5%とかなり発芽した。この種も産地間で差があり，発芽率は緯度，標高などとは関係がなく，変異した。キイチゴ属種子は埋土種子として報告があり，鈴木（1997）は8～9ヶ月間土に埋めた種子はよく発芽することを報告している。キイチゴ属の種子散布は，鳥やほ乳類による空間的な広がりと，埋土種子としての時間的な広がりの両方を散布戦術としているものと思われ，強い種子の休眠はそれと関係していると推測される。

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**Appendix**

Collection locality, latitude, altitude, date, collector and germination rate.

***Rubus crataegifolius*** (all in 1998)

**Miyazaki Pref.** : Saito-shi, Nishimiyakobara, 32°06′N, 60 m, May 19, I. Taki, 0%; Koyu-gun, Sintomi-cho, Shindenbara, 32°05′N, 60 m, May 9, I. Taki, 2%. **Tokushima Pref.** : Mima-gun, Mima-cho, Ryuoyama, 34°05′N, 730 m, May 31, O. Kume, 0%; Naka-gun, Kisawa-mura, Kitaura, 33°52′N, ca. 600 m, Jun. 30, T. Tabuchi, 0%. **Kagawa Pref.** : Ookawa-gun, Nagao-cho, Nyotai-yama, 34°12′N, 720 m, Jun. 4, O. Kume, 1%; Mitoyo-gun, Oonohara-cho, Gogo, Ebisai, Mandatoge, 34°00′N, 530 m, Jun. 6, O. Kume, 1%; Nakatado-gun, Chunan-cho, Shichika, Honme, Onoseyama, 34°10′N, 290 m, Jun. 6, O. Kume, 0%; Nakatado-gun, Kotonami-cho, Nakadori, Okawayama, 34°06′N, 650 m, Jun. 6, O. Kume, 0%. **Yamaguchi Pref.** : Saba-gun, Tokuji-cho, Notani, Shiraidani, 34°10′N, 200 m; Jun. 16; H. Masaki; 1%; Kuga-gun, Hongo-son,

Nishikurosawa, Hinourashimo, 34°17′N, 250 m, Jun. 29, H. Masaki, 0%; **Hiroshima Pref.** : Futami-gun, Mirasaka-cho, Haizuka, Hosodani, 34°47′N, ca. 300 m, Jun. 4, H. Ikeda et al., 0%; Futami-gun, Mirasaka-cho, Haizuka, Hosodani, 34°47′N, ca. 300 m, Jun. 14, H. Ikeda et al., 0%; Futami-gun, Mirasaka-cho, Haizuka, Hosodani, 34°47′N, ca. 300 m, Jun. 14, H. Ikeda et al., 0%. **Okayama Pref.** : Maniwa-gun, Kawakami-son, Kayabe, Kayabe-jinja, 35°15′N, 700 m, Jul. 12, H. Ikeda, 0%. **Aichi Pref.** : Minamishitara-gun, Tsukude-mura, Zenbu, 35°00′N, 540 m, Jun. 16, C. Suyama, 0%. **Gifu Pref.** :, Yoshiki-gun, Kamitakara-mura, Nakao, 36°18′N, 1100 m, Jul. 31, N. Naruhashi, 1%; Yoshiki-gun, Kamitakara-mura, Hirayu, Suki-jo, 36°10′N, 1,300 m, Jul. 31, N. Naruhashi, 0%; Masuda-gun, Osaka-cho, Nigorigo, 35°55′N, 1,700 m, Aug. 21, N. Naruhashi & K. Ban, 1%. **Ishikawa Pref.** : Kanazawa-shi, Shimizu-cho, 36°30′N, 200 m, Jun. 7, N. Naruhashi, 0%. **Toyama Pref.** : Shimoniikawa-gun, Oosawano-machi, Sarukurayama, 36°35′N, 220 m, Jun. 5, N. Naruhashi & K. Ban, 3%; Nei-gun, Yatsuo-machi, Muromaki-damu, 36°33′N, 260 m, Jun. 11, N. Naruhashi & K. Ban, 0%; Nei-gun, Yatsuo-machi, Uegashima, 36°33′N, 160 m, Jun. 11, N. Naruhashi & K. Ban, 0%; Nei-gun, Yamada-mura, Akamedani, 36°35′N, 300 m, Jun. 11; N. Naruhashi & K. Ban; 0%; Nei-gun, Fuchu-machi, Chisato, Jorakuji, 36°38′N, 100 m; Jun. 11, N. Naruhashi & K. Ban, 0%; Shimoniikawa-gun, Unazuki-machi, Unazuki, 36°48′N, 280 m, Jun. 23, N. Naruhashi & K. Ban, 0%; Shimoniikawa-gun, Unazuki-machi, Sogatake, 36°45′N, 430 m, Jun. 23, N. Naruhashi & K. Ban, 0%. **Akita Pref.** : Senboku-gun, Nishisenboku-machi, Kitanosawa, 39°35′N, 90 m, Jul. 7, Y. Horii, 1%; Kawabe-gun, Kawabe-machi, Kawakita-rindo, 39°40′N, 180 m, Jul. 7, Y. Horii, 6%; Senboku-gun, Oota-cho, Maki-keikoku, 39°30′N, 160 m, Jul. 22, Y. Horii, 0%; Senboku-gun, Oota-cho, Maki-keikoku, 39°30′N, 320 m, Jul. 22, Y. Horii, 0%.

***Rubus hirsutus*** (all in 1998)

**Miyazaki Pref.** : Saito-shi, Nishisaitobaru, 32°05′N, 60 m, May 19, I. Taki, 0%; Nishiusuki-gun, Hinokage-cho, Mitate, Okumura, 32°48′N, 600 m, May 10, T. Minamitani, 1%; Nishiusuki-gun, Hinokage-cho, Nanaore, Oosuge, 32°40′N, 150 m, May 10, T. Minamitani, 19%; Higashiusuki-gun, Shiiba-mura, Shimofukura, Ookubo, 32°28′N, 600 m, May 22, I. Taki, 0%. **Kumamoto Pref.** : Kikuchi-gun, Oozu-machi, Seta, 32°48′N, 250 m, May 13, J. Otobe, 4%; Kikuchi-gun, Oozu-machi, Muro, 32°48′N, 120 m, May 9, J. Otobe, 2%; Aso-gun, Choyo-mura, Kawayou, 32°48′N, 400 m, May 16, J. Otobe, 18%. **Tokushima Pref.** : Anan-shi, Kuwano-cho, Kuwanotani, 33°52′N, 60 m, May 23, T. Tabuchi, 6%; Katsuura-gun, Kamikatsu-cho, Boji, 33°50′N, 200 m, May 24, T. Tabuchi, 1%; Mima-gun, Mima-cho, Jyoyo, Aiguritoge, 34°05′N, 530 m, May 31, O. Kume, 0%; Mima-gun, Mima-cho, Sogo, 34°05′N, 590 m, May 31, O. Kume, 1%. **Kagawa Pref.** : Ayauta-gun, Ayakami-cho, Funshonishi, Shimoshinna, 34°10′N, 190 m, May 17, O. Kume, 9%; Mitoyo-gun, Oonohara-cho, Gogo, Ebisai, Mandatoge, 34°00′N, 530 m, May 30, O. Kume, 1%; Mitoyo-gun, Saita-cho, Saitakami, Haikura, 34°07′N, 250 m, May 30, O. Kume, 0%; Kagawa-gun, Shionoe-cho, Kaminishi, Matsuo, 34°07′N, 550 m, May 22, O. Kume, 15%; Nakatado-gun, Kotonami-cho, Nakadori, Ookawayama, 34°06′N, 630m, Jun. 6, O. Kume, 0%; Okawa-gun, Nagao-cho, Tawa, Kanewari, 34°12′N, 430 m, Jun. 4, O. Kume, 2%. **Yamaguchi Pref.** : Hagi-shi, Chinto, Tadokoyama, 34°25′N, ca. 100 m, May 23, His. Masaki, 0%; Kumage-gun, Hirao-cho, Saga, Jingayama, 33°52′N, 20 m, May 10, H. Masaki, 38%; Mine-gun, Shuho-cho, Iwanaga, Shimogo, 34°05′N, 110 m, May 15, H. Masaki, 0%; Toyoura-gun, Hohoku-cho, Awano, Saigase-Ichinose, 34°20′N, 25 m, May 15, H. Masaki, 8%; Kudamatsu-shi, Kamitoyoi, 33°55′N, 15 m, May 18, H. Masaki, 1%; Shinnanyo-shi, Eigenzan, 34°05′N, c. 60 m, May 17, His. Masaki, 20%; Shinnanyo-shi, Sengokudake, 34°07′N, ca. 540 m, May 17, His. Masaki, 3%; Yamaguchi-shi, Aihonijima, Kozaki, 33°55′N, ca. 10 m, May 22, His. Masaki, 0%. **Wakayama Pref.** : Gobo-shi, Maruyama, 33°54′N, ca. 30 m, May 10, K. Kinoshita, 5%. **Toyama Pref.** : Kami-niikawa-gun, Oosawano-machi, Sarukura, 36°35′N, 40 m, Jun. 5, N. Naruhashi & K. Ban, 6%; Kaminiikawa-gun, Oosawano-machi, Sarukura-yama, 36°35′N, 100 m, Jun. 5, N. Naruhashi & K. Ban, 0%; Kaminiikawa-gun, Oosawano-machi, Ushigamase, 36°35′N, 20 m, Jun. 5, N. Naruhashi & K. Ban, 1%; Himi-shi, Ao-shinrinkoen, 36°53′N, 10 m, Jun. 7, N. Naruhashi & S. Kojima, 1%.

***Rubus palmatus*** (all in 1999)

**Kagoshima Pref.** : Kagoshima-shi, Yoshino-cho, Terayama, 31°34′N, 380-400 m, May 31, S. Hosoyamada, 89%; Kagoshima-gun, Yoshida-cho, Mureigaoka, 31°42′N, 400-430 m, May 31, S. Hosoyamada, 82%; Izumi-shi, Shibisan, 31°57′N, ca. 1,000 m, Jun. 6, S. Hosoyamada, 50%. **Tokushima Pref.** : Tokushima-shi, Kamihachiman-cho, Bizan, 34°02′N, ca. 90 m, May 31, T. Tabuchi, 10%; Tokushima-shi, Takara-cho, Nakatsumine, 33°59′N, 500 m, Jun. 8, T. Tabuchi, 29%; Tokushima-shi, Takara-cho, Nakatsumine, 33°59′N, 600 m, Jun. 8, T. Tabuchi, 27%; Naka-gun, Kaminaka-cho, Furuyadani, 33°47′N, c. 350 m, May 28, T. Tabuchi, 2%; Katsuura-gun, Katsuura-cho, Baraotoge, 33°56′N, 450 m, Jun. 8, T. Tabuchi, 34%; Miyoshi-gun, Mino-cho, Tachinoyama, Ookawayama, 34°06′N, 920 m, Jun. 20, O. Kume, 27%; Mima-gun, Mima-cho, Fujiji, 34°04′N, 760 m, Jun. 20, O. Kume, 43%; Mima-gun, Mima-cho, Ryuoyama, 34°06′N, 1000 m, Jun. 20, O. Kume, 6%. **Kagawa Pref.** : Nakatado-gun, Chunan-cho, Sogo, Oiage, Shiroyama, 34°08′N, 290 m, Jun. 6, O. Kume, 20%; Nakatado-gun, Kotonami-cho, Nakadori, Hashitani, 34°08′N, 720m, Jun. 20, O. Kume, 1%. **Yamaguchi Pref.** : Hikari-shi, Miikannon,

33°55′N, 80 m, May 26, H. Masaki, 27%; Hikari-shi, Miikannon, 33°55′N, 80 m, May 30, H. Masaki, 66%; Yamaguchi-shi, Sayama, Sayamanishideguchi, 34°03′N, 50 m, Jun. 2, H. Masaki, 19%. **Hyogo Pref.** : Kobe-shi, Higashinada-ku, Uzumoridai, 34°45′N, 300 m, May. 30, M. Chikamori, 39%. **Ishikawa Pref.** : Fugeshi-gun, Monzen-machi, Nishimaruyama, 37°17′N, 300 m, Jun. 23, K. Umemoto, 44%; Fugeshi-gun, Monzen-machi, Futamatagawa, 37°16′N, 90 m, Jun. 20, K. Umemoto, 18%. **Toyama Pref.** : Himi-shi, Waki, 36°58′N, 50 m, Jun. 11, N. Naruhashi & K. Umemoto, 5%; Himi-shi, Nakanami, 36°58′N, 20 m, Jun. 11, N. Naruhashi & K. Umemoto, 2%; Himi-shi, Nakata, Taniguchi, 36°57′N, 50 m, Jun. 11, N. Naruhashi & K. Umemoto, 16%; Himi-shi, Sugata, 36°56′N, 30 m, Jun. 11, N. Naruhashi & K. Umemoto, 12%; Himi-shi, Isobe, Yatsushiro, 36°55′N, 100 m, Jun. 11, N. Naruhashi & K. Umemoto, 8%; Himi-shi, Kadoma, Nakataura, 36°56′N, 260 m, Jun. 11, N. Naruhashi & K. Umemoto, 31%; Himi-shi, Horita, 36°48′N, 30 m, Jun. 11, N. Naruhashi & K. Umemoto, 6%; Himi-shi, Nishiebisaka (Futagamisan), 36°47′N, 0 m, Jun. 11, N. Naruhashi & K. Umemoto, 34%; Nei-gun, Fuchu-machi, Sanya, Mitsuyabashi, 36°38′N, 80 m, Jun. 14, N. Naruhashi & A. Inomata, 1%; Nei-gun, Fuchu-machi, Minose, 36°37′N, 60 m, Jun. 14, N. Naruhashi & A. Inomata, 38%; Nei-gun, Fuchu-machi, Yoshitani, 36°38′N, 100 m, Jun. 14, N. Naruhashi & A. Inomata, 50%; Nei-gun, Yatsuo-machi, Shitanomyo, 36°34′N, ca.180 m, Jun. 19, K. Umemoto & A. Inomata, 21%; Nei-gun, Yatsuo-machi, Nunotani, 36°30′N, 170 m, Jun. 23, T. Yamada & A. Inomata, 7%; Nei-gun, Yatsuo-machi, Nishimatsuse, 36°30′N, 400 m, Jun. 23, T. Yamada & A. Inomata, 7%; Nei-gun, Yatsuo-machi, Myogajima, 36°31′N, 280m, Jun. 23, T. Yamada & A. Inomata, 87%; Nei-gun, Yatsuo-machi, Tochiore, 36°30′N, 340 m, Jun. 23, T. Yamada & A. Inomata, 39%; Nei-gun, Yatsuo-machi, Awasu, 36°30′N, 340 m, Jun. 23, T. Yamada & A. Inomata, 53%; Tonami-shi, Shogonji, 36°38′N, 60 m, Jun. 14, N. Naruhashi & A. Inomata, 25%; Tonami-shi, Seridani (Senkoji), 36°38′N, 90 m, Jun. 14, N. Naruhashi & A. Inomata, 1%; Imizu-gun, Kosugi-machi, Daira, 36°42′N, 270 m, Jun. 14, N. Naruhashi & A. Inomata, 6%; Kaminiikawa-gun, Oosawano-machi, Ishibuchi, 36°34′N, 280 m, Jun. 26, T. Yamada & A. Inomata, 48%; Shimoniikawa-gun, Asahi-machi, Ikenohara, 36°57′N, 250 m, Jun. 21, A. Inomata, 62%; Shimoniikawa-gun, Asahi-machi, Sasagawa, 36°57′N, 60 m, Jun. 15, A. Inomata, 14%; Shimoniikawa-gun, Asahi-machi, Iwasaki, 36°57′N, 200 m, Jun. 15, A. Inomata, 64%; Shimoniikawa-gun, Asahi-machi, Nakamura, 36°57′N, 210 m, Jun. 15, A. Inomata, 34%; Shimoniikawa-gun, Asahi-machi, Takabatake, 36°57′N, 210 m, Jun. 15, A. Inomata, 25%; Shimoniikawa-gun, Unazuki-machi, Sogatake, 36°46′N, 560 m, Jun. 28, T. Yamada, 3%; Shimoniikawa-gun, Asahi-machi, Yunose, 36°53′N, 390 m, Jun. 28, A. Inomata, 19%; Shimoniikawa-gun, Asahi-machi, Aimatadani, 36°53′N, 310 m, Jun. 28, A. Inomata, 27%. **Niigata Pref.** : Itoigawa-shi, Isohara, 37°02′N, 580 m, Jun. 14, K. Umemoto, 42%; Nishikubiki-gun, Nou-machi, Momokawa, 37°05′N, 20 m, Jun. 14, K. Umemoto, 47%; Nishikubiki-gun, Omi-machi, Yokoji, 37°02′N, 20 m, Jun. 14, K. Umemoto, 50%. **Akita Pref.** : Yokote-shi, Takinosawa, 39°20′N, c. 80 m, Jul. 9, Y. Horii, 4%.

***Rubus parvifolius*** (all in 1999)

**Kagoshima Pref.** : Kagoshima-shi, Yoshino-cho, Terayama, 31°39′N, 380 m, Jul. 17, S. Hosoyamada, 0%. **Yamaguchi Pref.** : Abu-gun, Abu-machi, Kaminagasawa, 34°34′ N, 460 m, Jul. 21, H. Masaki, 0%; Kuga-gun, Nishiki-cho, Rakanyama, 34°14′N, 820 m, Jul. 31, H. Masaki, 0%. **Hiroshima Pref.** : Aki-gun, Kumano-cho, Hiradani, 34°20′N, 280 m, Jul. 2, N. Tanaka, 0%. **Nara Pref.** : Yosino-gun, Higashiyosino-mura, Washiya, 34°25′N, 300-400 m, Jul. 16, H. Ueda, 0%. **Kyoto Pref.** : Otokuni-gun, Ooyamazaki-cho, Enmyouji,Wakiyama, 34°56′N, 10 m, Jul. 9, T. Takanashi, 0%. **Gifu Pref.** : Yoshiki-gun, Kamioka-cho, Shikama, 36°22′N, 730 m, Jul. 20, N. Naruhashi & M. Sugimoto, 0%; Yoshiki-gun, Kamitakara-mura, Kenza A, 36°17′N, 710 m, Jul. 20, N. Naruhashi & M. Sugimoto, 0%; Yoshiki-gun, Kamitakara-mura, Kenza B, 36°17′N, 710 m, Jul. 20, N. Naruhashi & M. Sugimoto, 0%; Oono-gun, Asahi-mura, Terasawa, 36°04′N, 760 m, Jul. 20, N. Naruhashi & M. Sugimoto, 0%; Oono-gun, Kuguno-cho, Koyana, 36°03′N, 620 m, Jul. 20, N. Naruhashi & M. Sugimoto, 0%. **Yamanashi Pref.** : Kitakoma-gun, Futaba-machi, 35°42′N, 360 m, Jul. 23, A. Inomata, 0%. **Nagano Pref.** : Kiso-gun, Kisofukushima-machi, Kosibata, 35°53′N, 770 m, Jul. 20, N. Naruhashi & M. Sugimoto, 0%; Kiso-gun, Kisofukushima-machi, Taguchi, 35°48′N, 770 m, Jul. 20, N. Naruhashi & M. Sugimoto, 0%; Kitaazumi-gun, Otari-mura, Sotozawa, 36°50′N, 780 m, Jul. 30, N. Naruhashi & A. Inomata, 0%; Kitaazumi-gun, Otari-mura, Tsuchikura, 36°50′N, 620 m, Jul. 30, N. Naruhashi & A. Inomata, 1%; Kitaazumi-gun, Otari-mura, Tanaka, 36°50′N, 720 m, Jul. 30, N. Naruhashi & A. Inomata, 0%; Kitaazumi-gun, Hakuba-mura, Kirikubo, 36°42′N, 680 m, Jul. 30, N. Naruhashi & A. Inomata, 9%. **Toyama Pref.** : Himi-shi, Kubo, 36°50′N, 0 m, Jul. 20, T. Yamada, 3%; Toyama-shi, Chaya-machi, 36°42′N, 60 m, Jul. 17, T. Yamada, 0%; Toyama-shi, Tera-machi, 36°42′N, 60 m, Jul. 17, T. Yamada, 0%; Toyama-shi, Nishinoban, 36°38′N, 0 m, Jul. 17, T. Yamada, 0%; Toyama-shi, Sugitani, 36°41′N, 20 m, Jul. 17, T. Yamada, 0%; Toyama-shi, Arisawa, 36°41′N, 5 m, Jul. 19, T. Yamada, 0%; Toyama-shi, Iwase, 36°46′N, 0 m, Jul. 22, T. Yamada, 3%; Shimoniikawa-gun, Asahi-machi, Yunose, 36°53′N, 430 m, Jul. 17, A. Inomata, 0%; Simoniikawa-gun, Asahi-machi, 36°56′N, 100 m, Jul. 28, N. Naruhashi et al., 2%: Simoniikawa-gun, Asahi-machi, Yunose, 36°52′N, 480 m, Jul. 28, N. Naruhashi et al., 3%; Simoniikawa-gun, Miyazakikaigan, 36°58′N, 0 m, Jul. 28, N. Naruhashi et al., 0%; Higashitonami-gun, Taira-mura, Nashitani, 36°24′N, 740 m, Jul. 31, T.

Yamada, 0%. **Niigata Pref.** : Itoigawa-shi, Kotaki, Nastunaka, 37°01′N, 290 m, Jul. 30, N. Naruhashi & A. Inomata, 0%; Itoigawa-shi, Yamanobo, 37°02′N, 520 m, Jul. 30, N. Naruhashi & A. Inomata, 0%. **Akita Pref.** : Oga-shi, Daijima, 39°54′N, 0 m, Jul. 25, Y. Horii, 0%; Yokote-shi, Kanazawanakano, 39°22′N, 70 m, Aug. 1, Y. Horii, 0%; Yokote-shi, Miroku, 39°21′N, 80 m, Aug. 10, Y. Fujita, 0%; Senboku-gun, Kyowa-cho, Sakai, 39°39′N, c. 80 m, Jul. 27, Y. Horii, 1%; Yuri-gun, Ouchi-machi, Yokoiwa, 39°26′N, 60 m, Jul. 29, Y. Horii, 0%; Kawabe-gun, Kawabe-machi, Kami, 39°40′N, 10 m, Aug. 2, Y. Horii, 0%; Senboku-gun, Nangai-mura, Nashinokida, 39°28′N, 20 m, Aug. 2, Y. Horii, 0%; Hiraka-gun, Sannai-mura, Ainono, 39°16′N, 90 m, Aug. 3, Y. Horii, 0%. **Hokkaido Pref.** : Otaru-shi, Shinko, 42°50′N, c. 10 m, Aug. 5, K. Kojima, 0%; Otaru-shi, Shinko, 5-chome, 42°50′N, c. 5 m, Aug. 9, K. Kojima, 0%; Hidaka-shicho, Shizunai-cho, Shizunai-kako, 42°20′N, 10 m, Aug. 20, Y. Horii, 0%.