園学雑. (J. Japan. Soc. Hort. Sci.) 75 (6): 463–468. 2006.

Effect of Mycorrhizal Inoculation and Activated Charcoal on Growth and Nutrition in Peach (*Prunus persica* Batsch) Seedlings Treated with Peach Root-Bark Extracts

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The effect of arbuscular mycorrhizal (AM) inoculation and activated charcoal on growth and nutrition in peach seedlings treated with peach root-bark extracts was studied under greenhouse conditions. Peach root-bark extracts significantly inhibited growth in both mycorrhizal and non-mycorrhizal seedlings, although mycorrhizal seedlings demonstrated better growth and biomass yield. Activated charcoal slightly alleviated the negative effects of root-bark extract treatment but reduced the benefits derived from mycorrhizal symbiosis. The initiation of mycorrhizal symbiosis may be delayed by activated charcoal through the adsorption of signal chemicals from host plants. Generally, mycorrhizal seedlings had better P and Ca nutrition. There were no differences in mycorrhizal infection among the inoculated plants, but there was increased sporulation in root-bark extract treatments without activated charcoal. These results suggest that activated charcoal should be applied after mycorrhizal symbiosis has been established.

Key Words: activated charcoal, arbuscular mycorrhiza, root-bark extracts, seedling growth.

Introduction

Peach attains fruit bearing age much earlier than most fruit tree species, but has the disadvantage of a short tree life (Mizutani, 1980). Consequently, frequent re-planting is necessary, and the gradual decline in tree vigor observed in replanted orchards, commonly referred to as 'replant failure', is a big problem in peach production.

Proebsting and Gilmore (1941) showed that the decomposition of plant remains from old trees might be responsible for the decline in seedling growth. It was observed that adding 500 g of peach roots to virgin soil used to grow peach seedlings significantly inhibited shoot growth and shoot weight relative to plants growing in untreated media.

Peach roots contain high levels of the cyanogenic glycoside prunasin, which, on hydrolysis, yields benzaldehyde and the toxic hydrocyanic (HCN) acid, while benzaldehyde is easily oxidized to benzoic acid. The toxicity of the decomposition products of prunasin, particularly cyanide, to peach has been demonstrated (Israel et al., 1973; Mizutani, 1980), and Mizutani et al. (1988) reported that condensed tannin-like substances extracted from peach roots have growth-inhibiting activity. Gur and Cohen (1988) have also reported an accumulation of heat resistant *Bacilli* capable of hydrolyzing prunasin as being responsible for the poor growth in replanted peach. The allelopathic effects of residual phytochemicals on growth and development in subsequent plantings have been broadly discussed by Rice (1984).

Arbuscular mycorrhizal (AM) fungi are soil microorganisms that form symbiotic relationships with a majority of cultivated plants. These fungi play an important role in the acquisition of nutrients from the soil by plants (Read, 1992). Mycorrhizal infection also improves plant tolerance to environmental stresses. Increased tolerance to drought has been reported in mycorrhizal plants probably due to increased water uptake via hyphal extraction (Davies et al., 1992), regulated stomatal conductance in response to hormonal signals (Drüge and Schönbeck, 1992), or by lowered leaf potential for greater turgor maintenance (Davies et al., 1993). Peach plants in association with AM fungi also suffer less damage from nematode infestation (Pinochet et al., 1995) and flooding (Rutto et al., 2002).

Activated charcoal, with its large surface area and ability to strongly adsorb organic compounds, is often used as a soil amendment for detoxification purposes. In particular, the ability of activated charcoal to remove residual herbicides from the soil solution has been widely investigated. Lamoreaux et al. (1989) reported the complete recovery of norflurazon, a fluorinated pyridizanone herbicide from water, soil, and sand matrixes by activated charcoal when applied at varying

Received; October 31, 2005. Accepted; May 31, 2006.
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concentrations. Ishii and Kadoya (1994) also reported that media amendment with charcoal has a positive effect on mycorrhizal symbiosis.

The objective of this study was to examine the effect that mycorrhizal inoculation and media amendment with activated charcoal has on the growth and nutrition of peach seedlings treated with peach root-bark extracts.

Materials and Methods

Seedling growth and inoculation with arbuscular mycorrhizal fungi

Stratified wild-form peach (*Prunus persica* Batsch) rootstock seeds were removed from the pits and sown in sterile vermiculite. After germination, 32 uniform seedlings were selected and each seedling was transplanted into a pot (16 cm diameter and 13 cm deep) filled with low-nutrient sandy soil sterilized by autoclaving at 121°C for 20 min. Half the pots were inoculated with 10 g each of a soil based *Gigaspora margarita* (Becker & Hall) inoculum (Central Glass, Tokyo, Japan), containing an average of 50 spores per gram. The seedlings were raised under greenhouse conditions and fertilized weekly with 200 mL per pot of Hoagland's mineral solution (Millner and Kitt, 1992) modified by halving the P concentration.

Preparation of peach root-bark extracts and activated charcoal suspension

Root bark collected from 18-year-old peach trees was dried in an oven to a constant weight and then ground into a fine powder. A ten-gram sample of the root-bark powder was extracted three times in 1000 mL of 80% ethanol for 24 h at 2°C. The three extracts were filtered and bulked before removing the ethanol fraction by evaporation *in vacuo*. The extract was made up to 100 mL with distilled water and kept as a stock solution. A flowable-non-sedimenting, activated charcoal suspension manufactured by Dainichi Chemicals (Osaka, Japan) was used in the experiment. The peach root-bark extract was diluted with water to 1000 ppm and applied three times a week at a rate of 100 mL per treated seedling. The charcoal suspension was diluted fifty times with distilled water as recommended by the manufacturer and applied monthly at a rate of 200 mL per seedling. Regular watering and weekly fertilization continued alongside the treatments, and control plants were drenched with similar amounts of water during treatment.

Seedling treatment

Seedlings were treated by drenching with the peach root-bark extract and charcoal suspension from two weeks after transplanting. The following four treatments were established for both mycorrhizal and non-mycorrhizal seedlings: i) Seedlings drenched with both the peach root-bark extract (RE) and activated charcoal (AC) suspension labeled + AC + RE; ii) RE only treatment (– AC + RE); iii) AC only treatment (+ AC – RE); and iv) non-treated controls (– AC – RE).

Seedling growth and biomass measurement

Shoot length was measured at the beginning of the experiment (at transplanting), and at seedling harvesting (three months later). Random samples were also collected from the non-mycorrhizal treatment to confirm the absence of mycorrhizal infection (data not shown). Shoot and root fresh weights were recorded immediately after harvesting and dry weights after oven drying at 80°C for 72 h.

Estimation of mycorrhizal infection and spore enumeration

Root samples were collected from the mycorrhizal treatment at the end of the experiment and stained to determine AM infection levels. Eighteen rootlets (1 ± 0.2 cm) per seedling were excised and cleared by autoclaving in 10% KOH before staining in 0.03% Chlorazol Black E (CBE) solution (Brundrett, 1994). The extent of root colonization was quantified under a compound microscope (× 200) using the grid-intersect method (Giovannetti and Mosse, 1980).

A portion of the sand media from all mycorrhizal pots was collected after seedling harvesting and oven dried to a constant weight. Spores were separated from 50 g units by density gradient centrifugation with 50% sucrose, trapped in stacked sieves with mesh sizes of 500 µm (above) and 125 µm (below) and rinsed into Petri dishes. The dishes were placed against a grid and all spores were counted under a dissecting microscope.

Shoot and root mineral analysis

Shoot and root samples collected at harvesting were digested by dry-ashing in a muffle furnace at 550°C for 5 h. The ash was taken up in 20% HCl, made up to 20 mL with distilled water, and used for analysis. Phosphorus was measured by blue colorimetry as molybdate-reactive P at 730 nm using a spectrophotometer (U-2001, Hitachi, Tokyo, Japan), and Ca and Mg were measured using an atomic absorption spectrophotometer (AA-6200, Shimadzu, Kyoto, Japan).

Starch analysis

Starch analysis was done colorimetrically using stem samples from four seedlings. Samples (50 mg) were added to 5 mL distilled water in test tubes and heated in a water bath for 5 min to ensure complete wetting. The tubes were cooled to room temperature before adding 5 mL sodium acetate buffer containing 15 U/mL of amyloglucosidase to each tube. The samples were incubated in a shaking water bath at 40°C for 1 h. The enzymatic reaction was terminated by placing the tubes in a boiling water bath for 5 min and then 4.9 mL of anthrone reagent was added to 0.1 mL of each sample and to glucose standards in clean tubes before transferring the samples and standards into a boiling

water bath for 15 min to speed up color development. Absorbance was measured at 620 nm using a spectrophotometer (U-2001) and the starch content was computed from readings obtained for glucose standards.

Results

Seedling growth

After three months, shoot length was significantly higher in the mycorrhizal − AC − RE and + AC − RE treatments and lowest in the non-mycorrhizal treatment without the charcoal amendment (− AC + RE) among all treatments (Fig. 1). There were no significant differences in growth between the mycorrhizal + AC + RE and − AC + RE treatments (Fig. 1, left side), and between the non-mycorrhizal − AC − RE and + AC + RE treatments on the other (Fig. 1, right side).

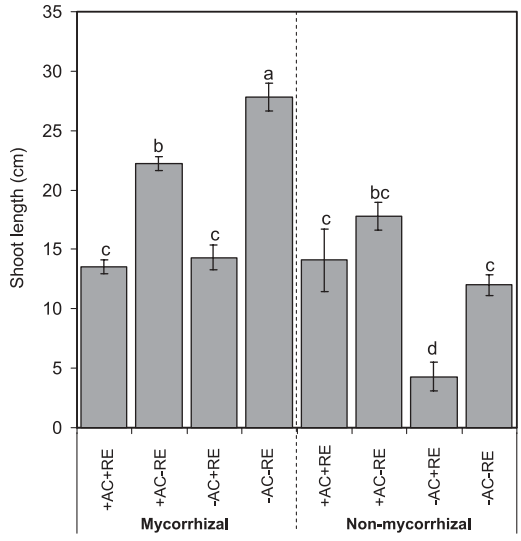

Fig. 1. Growth in mycorrhizal and non-mycorrhizal peach seedlings after transplanting into media with (+) or without (–) activated charcoal (AC) amendment and treatment with peach root-bark extracts (RE). Bars represent SE (n = 4), and different column letters show a significant difference at p < 5% (Tukey's multiple comparison test).

Seedling biomass

Shoot biomass weight was lower in both mycorrhizal and non-mycorrhizal seedlings treated with the root-bark extracts irrespective of activated charcoal amendment (Table 1). Except in the + AC − RE treatments where

Table 2. Shoot/root ratios in mycorrhizal and non-mycorrhizal peach seedlings grown in media amended with activated charcoal and peach root-bark extracts.

Treatment[z]			Shoot/root ratio	
AM	AC	RE	Fresh weight	Dry weight
+	+	+	0.69 ± 0.07[y]	0.96 ± 0.01
+	+	–	1.09 ± 0.07	1.35 ± 0.07
+	–	+	0.79 ± 0.06	1.06 ± 0.11
+	–	–	1.06 ± 0.04	1.31 ± 0.09
–	+	+	0.74 ± 0.14	1.21 ± 0.19
–	+	–	0.73 ± 0.11	1.28 ± 0.22
–	–	+	0.71 ± 0.06	1.08 ± 0.08
–	–	–	0.76 ± 0.05	1.54 ± 0.16

[z] Plus (+) or minus (–) mycorrhizal inoculation (AM), activated charcoal (AC), and root bark extracts (RE).
[y] Mean ± SE (n = 4).

Table 1. Fresh and dry biomass weights in mycorrhizal and non-mycorrhizal peach seedlings grown in media amended with activated charcoal and peach root-bark extracts.

Treatment[z]			Shoot weight (g)		Root weight (g)	
AM	AC	RE	Fresh	Dry	Fresh	Dry
+	+	+	6.42 ± 0.30 *ab*[y]	2.91 ± 0.19 *c*	9.43 ± 0.57 *a*	3.04 ± 0.16 *ab*
+	+	–	9.85 ± 0.32 *a*	4.63 ± 0.18 *ab*	9.11 ± 0.73 *a*	3.44 ± 0.16 *ab*
+	–	+	6.42 ± 0.22 *bc*	3.18 ± 0.13 *bc*	8.20 ± 0.37 *ab*	2.99 ± 0.15 *abc*
+	–	–	10.70 ± 0.41 *a*	5.14 ± 0.21 *c*	10.13 ± 0.23 *a*	3.95 ± 0.12 *a*
–	+	+	5.65 ± 1.04 *bc*	2.91 ± 0.46 *c*	7.77 ± 0.74 *ab*	2.41 ± 0.08 *bc*
–	+	–	6.72 ± 0.54 *b*	3.34 ± 0.10 *bc*	9.41 ± 1.01 *a*	2.77 ± 0.46 *abc*
–	–	+	3.83 ± 0.81 *c*	1.96 ± 0.47 *c*	5.48 ± 1.15 *b*	1.81 ± 0.40 *c*
–	–	–	5.10 ± 0.42 *bc*	2.22 ± 0.61 *c*	6.73 ± 0.65 *ab*	1.56 ± 0.52 *c*
Significance[x]						
AM			**	**	NS	**
RE			**	NS	NS	NS
AC			NS	NS	NS	NS
AM × RE			NS	NS	NS	NS
RE × AC			NS	NS	NS	NS
AM × AC			**	**	NS	NS
AM × RE × AC			NS	NS	NS	NS

[z] Plus (+) or minus (–) mycorrhizal inoculation (AM), activated charcoal (AC) and root bark extracts (RE).
[y] Column values are means ± SE and different letters within columns denote significant differences between treatments at p < 5% (n = 4) after ANOVA and mean separation with Tukey's multiple comparison test.
[x] NS: not significant; ** significant at p < 5%.

Table 3. Mineral content of root and shoot samples from mycorrhizal and non-mycorrhizal peach seedlings grown in media amended with activated charcoal and peach root-bark extracts. Data are presented only for mineral elements where significant differences were recorded.

Treatment[z]			Shoot P (%)	Root mineral content (%)		
AM	AC	RE		P	Ca	Mg
+	+	+	0.15 ± 0.01 *b*[y]	0.07 ± 0.00 *b*	0.13 ± 0.00 *a*	0.08 ± 0.00 *ab*
+	+	–	0.16 ± 0.00 *ab*	0.08 ± 0.01 *b*	0.12 ± 0.01 *ab*	0.05 ± 0.01 *b*
+	–	+	0.15 ± 0.01 *ab*	0.12 ± 0.01 *a*	0.09 ± 0.00 *b*	0.08 ± 0.01 *ab*
+	–	–	0.18 ± 0.00 *a*	0.14 ± 0.00 *a*	0.08 ± 0.00 *b*	0.08 ± 0.01 *ab*
–	+	+	0.09 ± 0.01 *c*	0.13 ± 0.01 *a*	0.09 ± 0.00 *b*	0.10 ± 0.01 *ab*
–	+	–	0.11 ± 0.01 *c*	0.14 ± 0.01 *a*	0.10 ± 0.01 *ab*	0.11 ± 0.01 *a*
–	–	+	0.10 ± 0.01 *c*	0.07 ± 0.00 *b*	0.11 ± 0.01 *ab*	0.06 ± 0.01 *b*
–	–	–	0.12 ± 0.01 *bc*	0.09 ± 0.01 *b*	0.10 ± 0.00 *ab*	0.07 ± 0.01 *ab*

[z] Plus (+) or minus (–) mycorrhizal inoculation (AM), activated charcoal (AC), and root bark extracts (RE).
[y] Column values are means ± SE and different letters within columns denote significant differences between treatments at $p < 5\%$ ($n = 4$) after ANOVA and mean separation with Tukey's multiple comparison test.

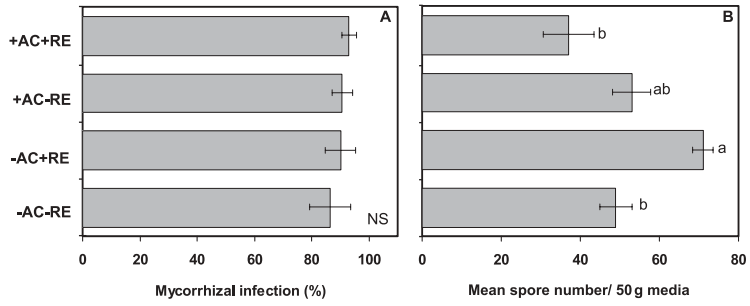

Fig. 2. Mycorrhizal infection (A) and extra radical spore number (B) in peach seedlings grown in media with (+) or without (–) activated charcoal (AC) amendment and treatment with peach root-bark extracts (RE). Bars represent SE ($n = 4$), and different column letters show a significant difference at $p < 5\%$ (Tukey's multiple comparison test). There were no significant differences (NS) between treatments in mycorrhizal infection.

root mass was higher in the non-mycorrhizal than in the mycorrhizal treatment, biomass production was higher in mycorrhizal relative to corresponding non-mycorrhizal treatments (Table 1).

With concomitant low shoot/root ratios, shoot growth was relatively lower in mycorrhizal seedlings treated with root-bark extracts and in treated non-mycorrhizal seedlings (Table 2).

Seedling shoot and root mineral contents

Shoot P was consistently higher in the mycorrhizal seedlings irrespective of charcoal amendment or treatment with root-bark extracts (Table 3). Root P was higher in non-mycorrhizal seedlings grown in the presence of activated charcoal, while the opposite was true for mycorrhizal seedlings. Overall, except for P, there was no marked difference in mineral acquisition between mycorrhizal and non-mycorrhizal seedlings (Table 3).

Starch content

Treatment with peach-root bark extracts had no significant effect on the starch content of seedling stems. Generally, starch content was relatively high in mycorrhizal seedlings with the exception of the + AC + RE treatment. It was highest in – AC + RE treatments for both mycorrhizal and non-mycorrhizal seedlings and lowest in mycorrhizal + AC + RE and non-mycorrhizal + AC – RE and – AC – RE treatments (Fig. 3).

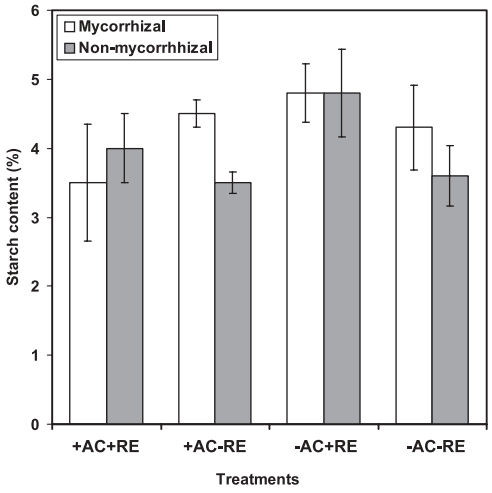

Fig. 3. Starch content in the stems of mycorrhizal and non-mycorrhizal peach seedlings grown in media with (+) or without (–) activated charcoal (AC) amendment and treatment with peach root-bark extracts (RE). Bars represent SE ($n = 4$). There were no significant differences in starch content between treatments.

Mycorrhizal infection, spore population, and extra-radical hyphal length

Treatment with peach root-bark extracts did not affect mycorrhizal infection in the presence or absence of activated charcoal. Mycorrhizal infection was consistently high (>80%) for all treatments (Fig. 2A). However, extra-radical sporulation was significantly higher in the – AC + RE relative to the other treatments (Fig. 2B).

Discussion

Roots and other plant detritus left to decay in the soil have been shown to inhibit growth in subsequent plantings (Proebsting and Gilmore, 1941). This allelopathic quality has been observed in peach, citrus, asparagus, and other crop species, and is possibly due to the release of phytotoxic substances over the growth period and upon decomposition (Patrick, 1971). Allelopathic effects on mycorrhiza have also been demonstrated where ethanol-soluble, heat-stable substances contained in litter of Scots pine (*Pinus sylvestris*) and various deciduous species were observed to inhibit ectomycorrhizal activity. Reduced AM formation in hardwood tree seedlings growing under pines has also been observed, and in one of our studies, we found that spore numbers and mycorrhizal infection levels in peach seedlings gradually increased along a transect away from the trunks of 18-year-old peach trees (unpublished data).

In the present experiment, it is clear that root-bark extracts inhibit peach seedling growth. Both mycorrhizal inoculation and amendment with activated charcoal slightly improved seedling performance relative to non-mycorrhizal seedlings growing in media without charcoal amendment. However, there is no evidence of a synergistic effect when AM inoculation and charcoal amendment are jointly applied. On the contrary, charcoal amendment resulted in a significant decline in the growth of mycorrhizal seedlings not treated with root-bark extracts (Fig. 1). Charcoal is useful as a soil amendment because of its ability to adsorb phytotoxic substances (Lamoreaux et al., 1989). However, in this study, activated charcoal is likely to have delayed the establishment of mycorrhizal symbiosis by adsorbing exudates that leak from respiring roots and play a critical role in the host/fungus signaling events that precede the establishment of symbiosis. There are reports that confirm the effectiveness of such exudates as stimulators of AM hyphal growth (Bécard and Piché, 1989; Gianinazzi-Pearson et al., 1989).

The data on biomass weights and shoot/root mass ratios (Tables 1 and 2) show that peach root-bark extracts inhibit shoot more than root growth in mycorrhizal seedlings. Low shoot/root ratios are common in non-mycorrhizal host plants that have to invest in additional root surface area to compensate for the absence of AM extra-radical hyphae. This was observed in this experiment for non-mycorrhizal seedlings whereby treatment with root-bark extracts resulted in a overall reduction in growth, but had no major effect on shoot/root ratios, whereas the low shoot/root ratios in mycorrhizal seedlings treated with root-bark extracts suggest that the mycorrhizal symbiosis may have been impaired (Fig. 1 and Table 2). However, the clear differences in shoot-P contents between mycorrhizal and non-mycorrhizal seedlings (Table 3) point to a normal symbiosis, implying that root-bark extracts exerted a direct effect on aboveground seedling growth. This could probably be through a disruption of long distance transport or interference with phytohormone mediated communication between below- and aboveground components.

Perry and Choquette (1987) suggest the possible scenarios of allelopathic AM inhibition as being either a direct inhibition of the AM symbiont or indirect inhibition through reduced host metabolism and health. The high AM infection levels observed in this experiment irrespective of root-bark extract treatment suggests that treatment with root-bark extracts may have exerted a greater influence on the plant host than on the fungus. In nature, allelopathic inhibition most often results from the combined action of several different chemicals. For example, several phenolic acids interfere with many physiological processes in higher plants (Einhellig, 1987). These disruptions include an alteration of plant water balance as in the depression of leaf water potential by ferulic and *p*-coumaric acids. These effects would interfere with the mycorrhizal symbiosis via limited photosynthate supply as a consequence of a decline in host metabolism.

Treatment with peach root-bark extracts does not seem to have a significant effect on root mineral content. However, an interesting observation is made in mycorrhizal and non-mycorrhizal seedlings where activated charcoal amendment results in a lower P level in mycorrhizal roots and vice versa (Table 3). This suggests that while charcoal improves P uptake in non-mycorrhizal roots, it might exert an opposite effect in mycorrhizal plants, probably through a negative interaction with the AM fungus.

Future research should attempt to identify the active chemical components contained in peach root-bark extracts and their mode of action.

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菌根菌接種と活性炭処理がモモの根樹皮抽出物を処理したモモ実生の成長と栄養に及ぼす効果

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根への菌根菌の接種と活性炭処理がモモの根樹皮抽出物を処理したモモ実生の成長と栄養に及ぼす効果と菌根菌の感染率，土壌における菌根菌の胞子密度に及ぼす効果について温室内で調査をした．菌根菌を接種した実生は接種していない実生に比べて生育が優れ，生育量が多かったが，モモの根樹皮抽出物を処理した区では，菌根菌の接種の有無にかかわらず実生の成長を抑制した．活性炭処理は根樹皮抽出物処理による成長に対する負の効果をわずかに軽減したが，菌根菌共生の効果を減少させた．それは活性炭が宿主植物から出される感染を引き起こすシグナル物質を吸着することによるのではないかと思われた．菌根菌を接種した実生では，P と Ca 含量が高くなる傾向が見られた．菌根菌を接種した場合，菌根菌の感染率は処理間に差が見られなかったが，活性炭処理をしない場合，根樹皮抽出物は胞子形成を促進した．これらの結果は，モモ根樹皮抽出物による生育抑制を軽減するには，まず菌根菌の共生を確立させてから，活性炭を処理するのが良いことを示唆している．