(2R)-butyl (7Z)-Dodecenoate, a Main Sex Pheromone Component of Illiberis (Primilliberis) pruni Dyar (Lepidoptera: Zygaenidae: Procridinae)?

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Abstract: Illiberis (Primilliberis) pruni Dyar (Lepidoptera: Zygaenidae) is an orchard pest distributed in China, Korea, the Far East of Russia, Mongolia and Japan. Field investigations organized in Naruto, Tokushima, Japan in 2008-2010 showed an attraction of the males of this species to (2R)-butyl (7Z)-dodecenoate alone and a mixture containing this compound and (2R)-butyl (9Z)-tetradecenoate. A further, more precise test, organized in 2011 clearly showed that for I. pruni males the most active attractant was the mixture containing (2R)-butyl (7Z)-dodecenoate and (2R)-butyl (9Z)-tetradecenoate in a ratio of 100:10. The seasonal flight of I. pruni as registered by catches in the pheromone traps in 2008-2011 was found to take place in June.

Key words: (2R)-butyl (7Z)-dodecenoate, (2R)-butyl (9Z)-tetradecenoate, sex attractant, seasonal flight

Introduction


In the secretions of the sex pheromone glands of females of Illiberis rotundata Jordan, Subchev et al. (2009) identified two biologically active components of the sex pheromone: (2R)-butyl (7Z)-dodecenoate (R-7-12) and (2R)-butyl (9Z)-tetradecenoate (R-9-14). Field tests showed that the optimal ratio of the two compounds for attracting males of the species was 30:100-50:100 (SUBCHEV et al. 2011). When testing synthetic compounds in the field (2008-2010), apart from I. rotundata, relatively small but steady catches of males of I. pruni were also registered in some of the traps. For further investigations concerning the attraction of I. pruni males to specific baits, additional field tests were organized in 2011.
In the present paper we report on the results of our field tests with *I. pruni* obtained during 2008-2011. We describe a new sex attractant for *I. pruni*, which we successfully used for a seasonal monitoring of the pest.

**Materials and Methods**

R-7-12 and R-9-14 used in the present investigations were synthesized as described earlier (Subchev et al. 2009). The enantiomeric purity of the two compounds was 98.5%, each of them also containing 2% of the corresponding E-isomer. The compounds, dissolved in hexane, were applied separately or as mixtures in various ratios onto grey rubber caps (0.7 cm in diameter). The baits prepared in such a way were wrapped in aluminum foil and kept in the refrigerator before use.

Field investigations were organized in cherry plots in Naruto, Tokushima, Japan. Details of the field work in 2008-2010 when R-7-12 and R-9-14 were applied separately, and mixtures containing different ratios of these two compounds were tested using sticky Delta traps, have been described (Subchev et al. 2009, Subchev et al. 2011). In 2011, only R-9-12 and 100:10 and 100:30 mixtures of R-7-12 and R-9-14 were tested because other ratios tested in 2008-2010 had shown no attractiveness to *I. pruni* males. In 2011, the three lures were tested in ten replicates at four sites in the region of Naruto University of Education and one site in mid-town in Naruto. The distance between the traps in a replicate was about four meters and varied from 10 to 3,200 m between the replicates. The traps were visited at 1-3 day intervals before the first catches and then checked and caught moths were counted and removed at 3-4 day intervals. The dates of trap setting and removal in the field each year are shown on Figs. 1-4.

Data from trap catches in 2008-2010 with different baits were analyzed non-parametrically by a Kruskal–Wallis analysis of variance followed by a Mann–Whitney U test for pair-wise comparisons. To establish any possible significant differences between catches in traps with different baits in 2011, analysis of variance (ANOVA) followed by a Student-Newman-Keuls test for multiple comparisons was used. Data on trap catches in 2011 were transformed to square root \([x + 0.5]\) before analysis. All statistical analyses were performed using SPSS 11.0.1 software program (SPSS 2001). The significant difference was set at \(P < 0.05\).

**Results**

During 2008-2009, when R-7-12 and R-9-14 separately and in seven different ratios were tested, the highest catches of *I. pruni* were observed in traps baited with a 100:10 mixture of the compounds. Some catches were registered in traps baited with only R-7-12 in both years, and in traps baited with a 100:30 mixture in 2008, while no moths were found in traps baited with other mixtures. The difference between the catches in traps baited with a 100:10 mixture and with R-7-12 separately was not significant, while the difference between the catches in these traps and the rest definitely was. In 2010, catches in the traps baited with R-7-12 were higher than in traps baited with the 100:10 mixture, but the
Fig. 2. Seasonal flight of *I. pruni* in Naruto, Tokushima, Japan monitored by pheromone traps in (a) 2008, (b) 2009, (c) 2010 and (d) 2011. Arrows (↓) show the date of the beginning and end of the test.
difference was not significant. In addition, there was a high standard error in catches using R-7-12 separately. Again, no insects were caught in the rest of the traps (Table 1). To avoid possible influences on the catches by the presence of too many pheromone sources, a field test with only the three treatments that had shown activity during 2008-2010 was organized in 2011. This test clearly showed that for *I. pruni* males the most active attractant was the mixture containing R-7-12 and R-9-14 in a ratio of 100:10 (Fig. 1).

The seasonal flight of *I. pruni* as registered by catches in the pheromone traps in 2008-2011 took place in June. The earliest catch was registered on June 1, 2009. Bearing in mind that *I. pruni* males are active in the afternoon, and the earliest visit was recorded on May 31 in the morning, the moth was most likely caught on May 31. The latest catches for all four years were registered on June 24 in both 2010 and 2011. The peak of the flight was in the middle of June. (Figs. 1-4)

### Discussion

Intensive investigations on the sex pheromone communication and the sex pheromone of *I. pruni* were carried out about 15 years ago by a group of Chinese scientists (*Li* et al., 1996a; *Li* et al., 1996b; *Li* et al. 1997a; *Li* et al. 1997b). Unfortunately all papers were published only in Chinese and in local journals and thus were largely overlooked by the scientific community. The first paper of this research group described the pheromone secretion and female calling behaviour in this species that was different from the calling behaviour known in other lepidopterans: *I. pruni* females bow their abdomen when they are calling (*Li* et al., 1996a). Observation of this calling posture of females was confirmed and documented with a photo by Nishihara, Wipking (2003). The same unusual calling behaviour for lepidopterans together with the description of the unusual location of the pheromone gland on the dorsal part of the abdomen was described in detail in another member of the zygaenid subfamily Procrinidae, *Theresimimma ampellophaga*, by Subchev, Harizanov (1990), Hallberg, Subchev (1997), and Toshova, Subchev (2003). Further investigations have shown that this calling behaviour is common in many other Procrinidae species (*Efetov 2001, Toshova, Subchev 2005*) and most likely is an autapomorphic character of the subfamily Procrinidae (*Subchev 2003*).

Later, using GC-EAD and further MS analysis *Li* et al. (1997a) described the active compound present in *I. pruni* as a butyl dodecenoate. However, to the best of our knowledge no detailed structure assignment of the compound and/or its synthesis has been published. When comparing the published mass spectrum of the natural butyl dodecenoate (*Li* et al. 1997a) and the mass spectrum of synthetic R-7-12 (*Subchev et al. 2009*) a full coincidence was found. The latter fact and the activity of R-7-12 as an attractant for *I. pruni* males reported in the present paper strongly suggests that this compound is a main sex pheromone component in this species. According to our field results, and especially to those obtained in 2011, *I. pruni* seems to use R-7-12 as a main sex attractant and R-9-14 as a minor component acting as a synergist to R-7-12 when applied as a 100:10 admixture.

### Table 1

Mean catches of *I. pruni* in traps baited with R-7-12 separately, R-9-14 separately and seven other mixtures of these compounds in Naruto, Tokushima, Japan during 2008-2010. Numbers marked with the same letter in a row are not significantly different at *P* < 0.05, Kruskal–Wallis analysis of variance followed by a Mann–Whitney U test for pair-wise comparisons.

<table>
<thead>
<tr>
<th>Year</th>
<th>(2R)-butyl (7Z)-dodecenoate : (2R)-butyl (9Z)-tetradecenoate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100:0</td>
</tr>
<tr>
<td>2008</td>
<td>0.33ab±0.59</td>
</tr>
<tr>
<td>2009</td>
<td>0.29a±0.55</td>
</tr>
<tr>
<td>2010</td>
<td>0.54a±1.35</td>
</tr>
</tbody>
</table>
As already mentioned, R-7-12 and R-9-14 are sex pheromone components of *I. rotundata*. Males of this species are attracted in the field only by a mixture of these two compounds, the best ratio being 30:100 – 50:100, but not by the compounds applied separately (Subchev et al. 2009, Subchev et al. 2011). *I. pruni* and *I. rotundata* are closely related species (Efetov, Tarman 1995) with overlapping daily and seasonal flight. Thus the main isolating factor preventing cross attraction of the males by conspecific females seems to be the different ratio of R-7-12 and R-9-14 shared by the two species as pheromone components with the predominance of R-7-12 in the case of *I. pruni* and R-9-14 in the case of *I. rotundata*. According to Nishihara, Wipking (2003) *I. pruni* is univoltine, and adults emerge from late May to early July but the emergence period shows regional and annual variation. Their own studies in areas in Nagoya city confirmed this and in addition they found that the peak of emergence is the middle of June (Nishihara, Wipking, 2003). Esaki (1971) stated that in Japan *I. pruni* adults emerged in June while Oku (2003) reported for this pest an emergence time of late June to early July in the Tohoku area and mainly June in Kanto. Thus, our results based on precise regular observations by pheromone traps during a period of four years confirmed that in Japan, and Tokushima in particular, *I. pruni* flies from late May to late June with a peak in mid June.

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