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TECHNICAL NOTE

Alternate lighting to enhance the oviposition behavior of parasitoid wasps
by increasing the movement of host larvae

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Abstract

An optical method, alternate lighting, is proposed to increase the oviposition activity of parasitoid wasps. The Mediterranean flour moth *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) is a substitute host for rearing *Meteorus pulchricornis* (Wesmael) (Hymenoptera: Braconidae), a parasitoid wasp of exposed-living lepidopteran pests, but its low autonomous oviposition in *E. kuehniella* larvae had to be improved. We

attempted to enhance the oviposition behavior of *M. pulchricornis* by increasing the movement of *E. kuehniella* larvae through negative phototaxis by alternate LED lighting. Alternate lighting at 5 min intervals approximately tripled the movement of *E. kuehniella* larvae and almost doubled the number of eggs laid by *M. pulchricornis* into them. Silk-thread entanglement was a critical problem that decreased intact wasps when they were together with *E. kuehniella* larvae; however, it could be relieved under alternate lighting.

Key words *Ephestia kuehniella* • LED • *Meteorus pulchricornis* • negative phototaxis • optical manipulation • silk-thread entanglement

Introduction

High oviposition rate is essential to efficient mass-rearing systems of parasitoids for augmentative biological control. For that purpose, here, we propose an optical method to increase the oviposition activity of parasitoid wasps.

Meteorus pulchricornis (Wesmael) (Hymenoptera: Braconidae) is a solitary koinobiont endoparasitoid of exposed-living lepidopteran larvae (Maeto 1989, 2018), and is a common natural enemy of *Helicoverpa*, *Mamestra*, *Spodoptera* (Lepidoptera: Noctuidae), *Lymantria*, *Orgyia* (Erebidae), and other pest herbivores (e.g., Liu and Li 2006, 2008; Marsh 1979; Takashino et al. 1998; Walker et al. 2016). Owing to its wide host range (Maeto 2018) and clonal reproduction (Tsutsui et al. 2014), *M. pulchricornis* could be a potential biocontrol agent for various lepidopteran pests. Nakano et al. (2018) have demonstrated that this parasitoid can be successfully reared on the larvae of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) as a substitute host for mass-rearing. In their experiments, however, egg deposition by *M. pulchricornis* into *E. kuehniella* larvae was artificially induced by shaking a larva in front of a wasp. Autonomous oviposition of *M.*

pulchricornis on the larvae of *E. kuehniella* had to be confirmed first and enhanced to a feasible extent.

The natural hosts of *M. pulchricornis* are caterpillars moving freely on plants, and its oviposition behavior is triggered by the movement of host larvae (Yamamoto et al. 2009). However, the larvae of *E. kuehniella* like to conceal themselves among dry plant materials sewed with silk threads, due to negative phototaxis (Brandt 1934). Therefore, the larvae should be removed from food materials and stimulated to become active as much as possible for the oviposition behavior of wasps. Another critical problem is that female adults of *M. pulchricornis* often get entangled in the silk threads spun by the larvae of *E. kuehniella* during oviposition.

In this study, we aimed to enhance the autonomous oviposition of *M. pulchricornis* in *E. kuehniella* larvae by optical manipulation. The moving of *E. kuehniella* larvae was accelerated with alternate LED lighting, by utilizing the negative phototaxis of the larvae (Brandt 1934; Shimoda and Honda 2013), in order to increase the oviposition activity of *M. pulchricornis* into them. Also, a feasible length of time for oviposition was determined to avoid entanglement in the silk threads of *E. kuehniella*.

Materials and methods

Insects

A strain of *E. kuehniella* donated by the Western Region Agricultural Research Center (WARC/NARO, Japan) was reared on pressed corn at 25°C under a 16L:8D photoperiod (16 h light and 8 h dark). For all the experiments, late-instar larvae (fresh weight: 10.0 - 40.0 mg) were provided as hosts.

A thelytokous strain of *M. pulchricornis* originating from *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) in Japan (Nguyen et al. 2005; strain code

KAGAWAZ_01_01 of Fujie et al. 2019) was used in experiments 2 and 3. It was maintained on *S. litura* larvae reared on artificial diets (Insecta LFS, Nosan Corporation, Yokohama, Japan). Adult wasps were given an absorbent cotton ball soaked in a 50% honey solution and kept under a 16L:8D photoperiod at 15°C in order to prolong the lifespan of the wasps. Adult wasps of less than three-week-old were used in the experiments.

Experimental device

Two LED lights (400 – 700 nm, peak 450 nm) (CLEAR LED POWER III 600, GEX Corporation, Osaka, Japan) were set up along the upper lateral edges of the outer cardboard container (Fig. 1). At most eight transparent plastic cases (150 × 24 × 24 mm, Clear pen case, Lindexs, Shanghai, China) for enclosing insects for examination were set in parallel on the bottom of the container. A black, lightproof plastic plate was placed in the middle of the container to reduce light spread. Video recordings were obtained with a web camera (1280 × 720 pixels, ELP-USBHD06H-FV, Ailipu Technology Corporation, Shenzhen, China) set on top of the outer container. This experimental device was placed in a dark room at 25°C.

The two LED lights were made to emit light simultaneously in the condition of constant lighting, while one of them was made to emit light in turns in the condition of alternate lighting. In constant lighting, the photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$, average $\pm$ SD, $n = 15$) at the both ends and the center of the plastic cases was 35.56 ± 1.28 and 37.52 ± 1.53 , respectively. In alternate lighting, that at the light end, the center, and the dark end of the plastic cases was 35.45 ± 1.32 , 19.00 ± 1.28 , and 0.08 ± 0.00 , respectively. The photon flux density was measured by LI-190SA Quantum Sensor and LI-250A Light Meter (LI-COR Biosciences, Lincoln, Nebraska, USA).

Experiment 1: to increase the mobility of *E. kuehniella* larvae by alternate lighting

One *E. kuehniella* larva was put in the transparent case for 24 h. Two lighting conditions, constant and alternate at 5 min intervals, were provided to compare the mobility of larvae. The total length of larval movement in 24 h was measured by the visual observation of video recordings. For the constant and alternate lighting conditions, 21 and 25 larvae were provided, respectively. It was compared between constant and alternate lighting conditions by the generalized linear models (GLM), implemented in IBM SPSS Statistics 25 (IBM Corporation, Armonk, New York, USA), with the gamma distribution and log-link function.

Experiment 2: to estimate the timespan free from silk-thread entanglement

One wasp and one *E. kuehniella* larva were put together in the transparent case for 24 h. Two lighting conditions, constant and alternate 5 min intervals, were provided for the observation of the wasp's movement. The time (in hours) when a wasp became crippled, mainly due to silk-thread entanglement, was determined by the visual observation of video recordings. For the constant and alternate lighting conditions, 35 and 51 cases were examined, respectively. Overall proportion of intact (non-crippled) wasps was tested between constant and alternate lighting conditions using the log-rank test, implemented in IBM SPSS Statistics 25.

Experiment 3: to increase oviposition on *E. kuehniella* larvae by alternate lighting

One wasp and one *E. kuehniella* larva were put together for oviposition in the transparent case for 6 h, because the proportion of intact wasps had decreased markedly after that as shown in experiment 2. The wasps were moved into the experimental device from the

rearing room (16L:8D) two hours before the start time of scotophase in the room, because adult *M. pulchricornis* is known to have the circadian rhythm with a peak of activity around the beginning of scotophase (Nishimura et al. 2015).

Two lighting conditions, constant and alternate at 5 min intervals, were provided for the observation of wasp oviposition. After 6 h the wasp and larva were removed from the case, and the larva was reared with pressed corn in a transparent plastic case (40 mm diameter $\times$ 25 mm height). After three days, the larva was dissected in 70% ethanol under a stereoscopic microscope in order to count the number of eggs or hatched larvae of wasps.

A total of 24 wasps were provided after being trained for oviposition by giving them ten *S. litura* larvae together for two days beforehand. They were repeatedly used after two or more days of rest. Fresh weight of host larvae were measured by an analytical balance (CPA64, Sartorius AG, Göttingen, Germany) just before experiments.

The number of eggs laid (including hatched larvae) was compared between constant and alternate light conditions by the generalized linear mixed models (GLMM), implemented in IBM SPSS Statistics 25, with the Poisson distribution and log-link function. Light conditions, the weight of host larvae, and their interaction were tested as fixed factors, and individual wasps were treated as a random factor because they were used repeatedly (as shown in Table S3).

Results and discussion

Under alternate lighting, *E. kuehniella* larvae were often observed moving from the light side to the dark side, indicating negative phototaxis (Supplementary Video 1, 64x times speed). The mobility of larvae was significantly different between constant and alternate lighting conditions (GLM, *Wald Chi-Square* = 32.6, *df* = 1, *p* < 0.001) (Table S1). The estimated marginal mean (95% Wald Confidence Interval) of the total length of larval

movement in 24 h under constant lighting was 509.4 cm (379.2 – 684.5 cm) (21 larvae) and that under alternate lighting at 5 min intervals was 1638.0 cm (1249.6 – 2147.2 cm) (25 larvae) (Fig. 2). Alternate lighting approximately tripled the movement of *E. kuehniella* larvae.

The proportion of intact wasps of *M. pulchricornis* decreased mainly due to them being crippled by silk-thread entanglement (Supplementary Video 2). Wasps, once crippled, hardly ever recovered. The decrease was marked after about 10 h especially under constant lighting (Fig. 3) (Table S2). Overall proportion of intact wasps was significantly different between constant lighting and alternate lighting (log-rank test, *Chi-square* = 5.84, *df* = 1, *p* = 0.016).

Figure 4 shows the number of *M. pulchricornis* eggs (including hatched larvae) laid in 6 h under constant and alternate lighting on the fresh weight of *E. kuehniella* larvae (Table S3). In the first analysis of GLMM, the interaction of light conditions and the weight of host larvae on the number of eggs was not significant ($F = 2.05$, *df* = 1, 89, *p* = 0.156), and therefore the analysis was re-conducted only on the main effects. Then, the effect of light conditions on the number of eggs was significant ($F = 10.36$, *df* = 1, 90, *p* = 0.002), while that of the weight of host larvae was not significant ($F = 0.02$, *df* = 1, 90, *p* = 0.895). The estimated marginal mean (95% Confidence Interval) of the number of eggs laid per wasp in 6 h under constant lighting was 0.714 (0.492 – 1.035) (44 cases) and that under alternate lighting at 5 min intervals was 1.421 (1.091 – 1.850) (49 cases) when the weight of host larvae was fixed at 22.0 mg. Alternate lighting almost doubled the number of eggs laid into *E. kuehniella* larvae.

Our study shows that autonomous oviposition by larval parasitoids into substitute hosts can be enhanced by the optical method. According to Yamamoto et al. (2009), it is expected that increased host movement accelerates the oviposition behavior of *M.*

pulchricornis. In fact, the number of eggs laid by the wasp could be increased by the enhancement of host larval movement under alternate LED lighting. The mobility of host larvae, *E. kuehniella*, seems to be boosted under alternate lighting due to its negative phototaxis (Brandt 1934).

On the other hand, positive phototaxis of wasps may also have increased the chance of them coming into contact with host larvae under alternate lighting. The positive phototaxis is known in some chalcidoid parasitoids (Van Alebeek et al. 2007; Niedermayer et al. 2016), while not yet in the present wasp *M. pulchricornis*.

Although the number of eggs laid per wasp was up to 1.42 in 6 h (i.e., about 5.7 eggs per day) under alternate lighting, this is still about the half of potential daily fecundity of this species, that is about 12 eggs (Fuester et al. 1993). In our oviposition experiment, only one host larva was provided to a wasp. However, *M. pulchricornis* females could induce a short-term host paralysis and this may cause host larvae to interrupt movement after oviposition and decrease the chance of being oviposited again (Chau and Maeto 2009). Also, according to Sheng et al. (2014), a high host density has a positive effect on the host searching behavior of *M. pulchricornis*. Therefore, oviposition behavior could be activated more by providing multiple hosts to each wasp.

As observed in experiment 2, *M. pulchricornis* wasps were often crippled by the silk-threads spun by *E. kuehniella* larvae. This is likely because that this parasitoid attacks naked larvae on plant bodies in nature but not larvae hidden in webbed grains. However, the decrease of intact wasps owing to the silk-thread entanglement was markedly slow under alternate lighting (Fig. 3), probably because the time of host larvae for spinning threads could decrease while the time for moving increased under alternate lighting. Silk-thread entanglement may be avoided by conducting oviposition within several hours under alternate lighting, whereas the effects of lighting on spinning behavior of host

larvae *E. kuehniella* should be clarified.

Alternate lighting may be also considered for enhancing the oviposition activity of any other parasitoids that attack free-living hosts having negative or positive phototaxis.

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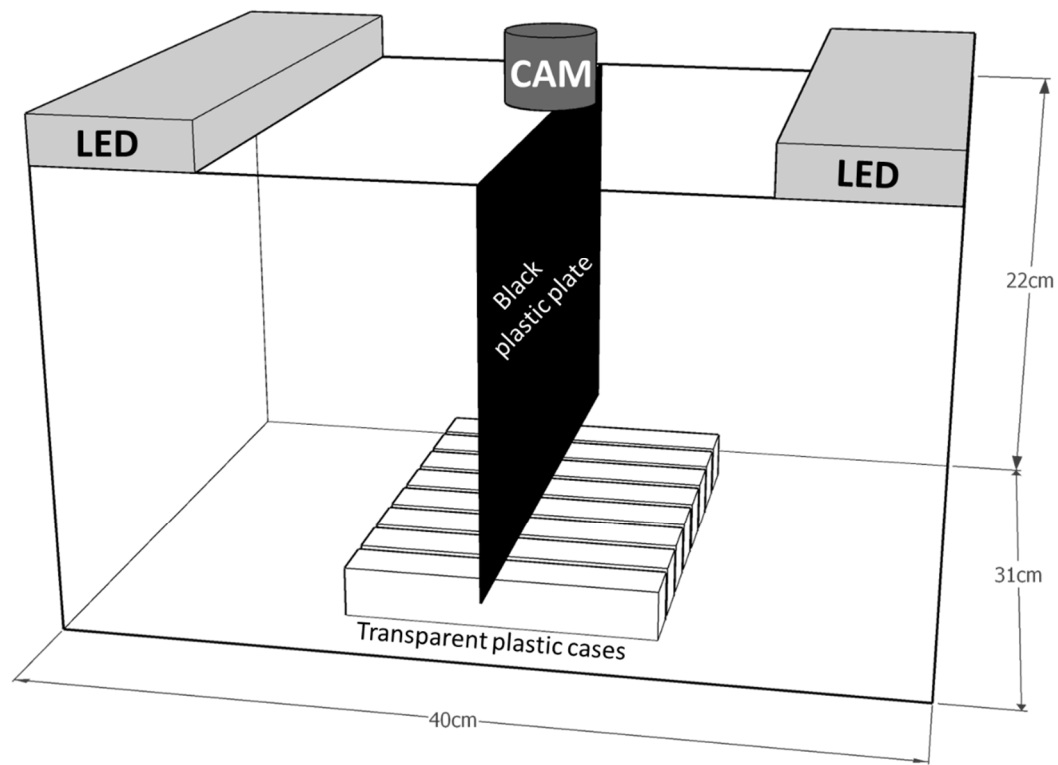


Fig. 1 Set-up of the experimental device. Two LED lights were set up along the upper lateral edges of the outer cardboard container. At most eight transparent plastic cases for enclosing insects were set in parallel on the bottom. A black, lightproof plastic plate was placed in the middle of the container to reduce light spreading.

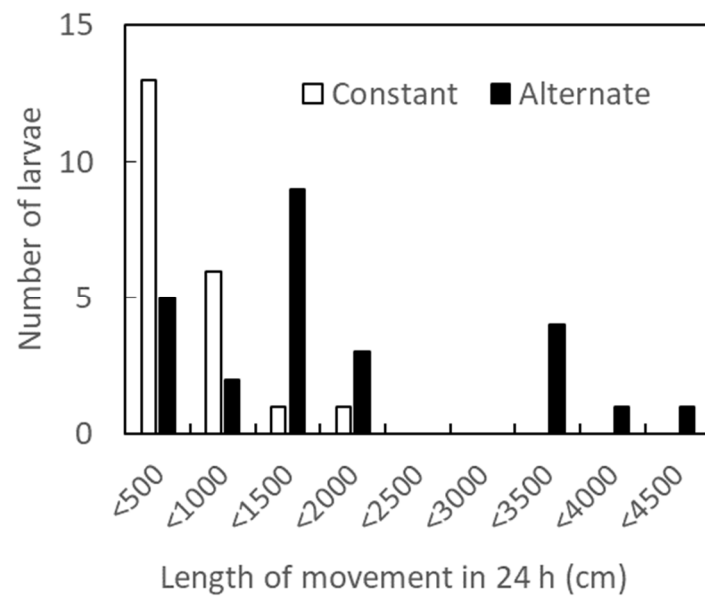


Fig. 2 Total length of the movement of *E. kuehniella* larvae in 24 h under constant lighting and alternate lighting at 5 min intervals.

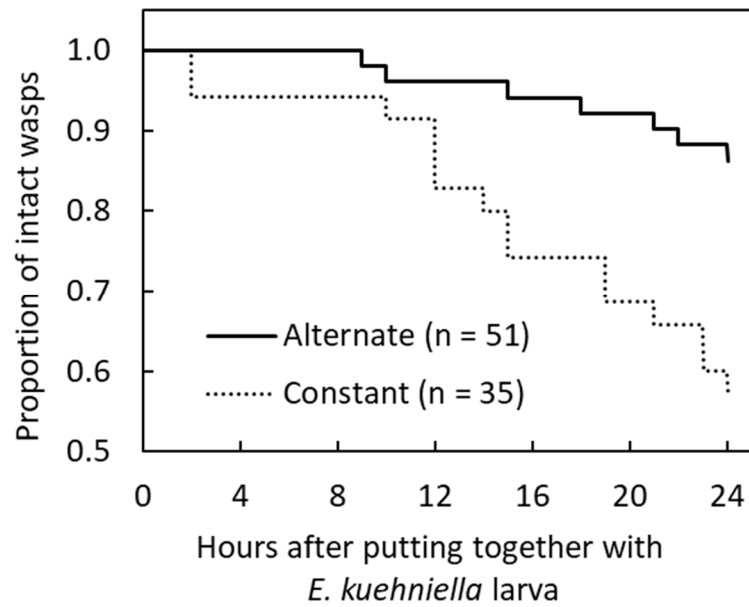


Fig. 3 Temporal changes in the proportion of intact (non-crippled) *M. pulchricornis* wasps together with *E. kuehniella* larva under constant lighting and alternate lighting at 5 min intervals.

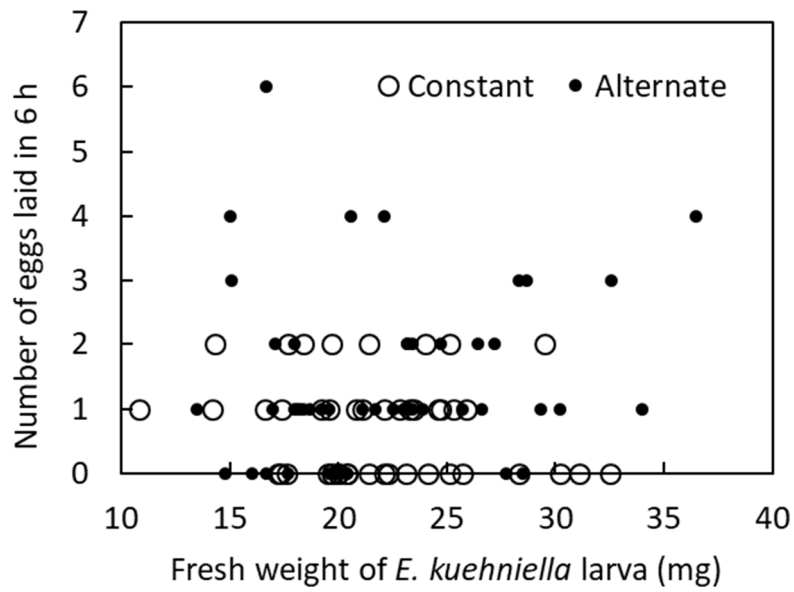


Fig. 4 The number of *M. pulchricornis* eggs (including hatched larvae) laid in 6 h under constant lighting and alternate lighting at 5 min intervals on the fresh weight of *E. kuehniella* larvae.

Table S1 Total length of movement of *E. kuehniella* larvae in 24 h

Lighting	Year/Month/Date	Length of movement (cm)
Constant	2019/4/17	205.2
Constant	2019/4/17	661.8
Constant	2019/4/17	516.9
Constant	2019/4/17	239.4
Constant	2019/5/5	219.3
Constant	2019/5/5	339.6
Constant	2019/5/5	1727.4
Constant	2019/7/2	1101
Constant	2019/7/2	949.8
Constant	2019/11/15	126.6
Constant	2019/11/15	493.2
Constant	2019/11/15	418.2
Constant	2019/11/15	502.8
Constant	2019/11/18	498
Constant	2019/11/18	90
Constant	2019/11/18	571.2
Constant	2019/11/18	285.6
Constant	2020/3/3	186
Constant	2020/3/3	902.4
Constant	2020/3/3	342
Constant	2020/3/3	321.6
Alternate	2019/4/29	1436.7
Alternate	2019/4/29	1136.4

Alternate	2019/4/29	4031.7
Alternate	2019/4/29	3211.8
Alternate	2019/5/3	405.9
Alternate	2019/5/3	3450
Alternate	2019/5/3	253.8
Alternate	2019/5/10	3942
Alternate	2019/5/10	1141.8
Alternate	2019/5/10	1371
Alternate	2019/5/10	1682.4
Alternate	2019/5/12	1332
Alternate	2019/5/12	1453.8
Alternate	2019/5/12	1245.6
Alternate	2019/5/12	1528.2
Alternate	2019/6/18	616.8
Alternate	2019/6/18	1731.3
Alternate	2019/6/18	1324.2
Alternate	2019/6/18	3168
Alternate	2019/6/27	3147.6
Alternate	2019/6/27	1393.8
Alternate	2019/6/27	651
Alternate	2019/6/27	418.2
Alternate	2019/11/16	442.8
Alternate	2019/11/16	433.2

Table S2 Number of intact wasps after putting together with an *E. kuehniella* larva

Hours	Constant lighting	Alternate lighting at 5 min intervals
0	35	51
1	35	51
2	33	51
3	33	51
4	33	51
5	33	51
6	33	51
7	33	51
8	33	51
9	33	50
10	32	49
11	32	49
12	29	49
13	29	49
14	28	49
15	26	48
16	26	48
17	26	48
18	26	47
19	24	47
20	24	47
21	23	46

22	23	45
23	21	45
24	20	44

Table S3 Number of wasp eggs laid in 6 h

Lighting	Year/Month/Date	Wasp	Host weight (mg)	Number of eggs (including hatched larvae)
Constant	2019/12/30	R23	17.7	2
Constant	2020/1/6	R25	22.3	0
Constant	2020/1/6	R26	25.7	0
Constant	2020/1/6	R28	30.2	0
Constant	2020/1/6	R30	22.1	1
Constant	2020/1/6	R31	19.6	1
Constant	2020/1/9	R26	21.4	2
Constant	2020/1/9	R28	17.3	0
Constant	2020/1/9	R30	19.7	0
Constant	2020/1/9	R31	21.1	1
Constant	2020/1/17	R26	19.2	1
Constant	2020/1/17	R28	18.4	2
Constant	2020/1/17	R30	19.7	2
Constant	2020/1/17	R31	19.5	0
Constant	2020/1/20	R33	21.4	0
Constant	2020/1/20	R34	23.3	1
Constant	2020/1/30	R52	17.4	1
Constant	2020/1/30	R56	17.6	0

Constant	2020/1/30	R57	10.8	1
Constant	2020/2/15	R77	24.1	0
Constant	2020/2/15	R78	20.8	1
Constant	2020/2/15	R79	25.9	1
Constant	2020/2/15	R80	25.1	0
Constant	2020/2/15	R81	16.6	1
Constant	2020/2/15	R82	24.6	1
Constant	2020/2/15	R83	24.7	1
Constant	2020/2/17	R70	29.5	2
Constant	2020/2/17	R71	25.1	2
Constant	2020/2/17	R72	23.1	0
Constant	2020/2/17	R74	28.3	0
Constant	2020/2/17	R76	14.2	1
Constant	2020/3/9	R70	31.1	0
Constant	2020/3/9	R71	24	2
Constant	2020/3/9	R72	32.5	0
Constant	2020/3/9	R76	17.2	0
Constant	2020/3/9	R79	25.3	1
Constant	2020/3/9	R81	22.1	0
Constant	2020/3/9	R83	20	0
Constant	2020/3/10	R72	23.5	1
Constant	2020/3/10	R76	20.4	0
Constant	2020/3/10	R78	20	0
Constant	2020/3/10	R79	14.3	2
Constant	2020/3/10	R81	20.4	0

Constant	2020/3/10	R83	22.8	1
Alternate	2020/1/1	R18	22.5	1
Alternate	2020/1/1	R23	18	2
Alternate	2020/1/4	R25	23.4	1
Alternate	2020/1/4	R26	25.7	1
Alternate	2020/1/4	R28	30.2	1
Alternate	2020/1/4	R30	22.1	4
Alternate	2020/1/4	R31	19.6	0
Alternate	2020/1/8	R25	19.8	0
Alternate	2020/1/8	R26	28.5	0
Alternate	2020/1/8	R28	18.2	1
Alternate	2020/1/8	R30	18	1
Alternate	2020/1/8	R31	20.4	0
Alternate	2020/1/12	R33	23.2	2
Alternate	2020/1/12	R34	18.7	1
Alternate	2020/1/16	R26	26.4	2
Alternate	2020/1/16	R28	20.4	0
Alternate	2020/1/16	R30	24.7	2
Alternate	2020/1/16	R31	21.1	1
Alternate	2020/1/22	R52	23	1
Alternate	2020/1/22	R56	23.9	1
Alternate	2020/1/22	R57	20.1	0
Alternate	2020/1/23	R26	20.6	4
Alternate	2020/1/23	R30	19.6	1
Alternate	2020/1/28	R52	29.3	1

Alternate	2020/1/28	R54	17	1
Alternate	2020/1/28	R56	17.7	0
Alternate	2020/1/28	R57	16.7	0
Alternate	2020/2/12	R70	26.6	1
Alternate	2020/2/12	R71	27.7	0
Alternate	2020/2/12	R72	21.7	1
Alternate	2020/2/12	R74	18.4	1
Alternate	2020/2/12	R76	15	4
Alternate	2020/2/20	R77	28.3	3
Alternate	2020/2/20	R78	23.2	2
Alternate	2020/2/20	R79	23.3	1
Alternate	2020/2/20	R80	19.2	1
Alternate	2020/2/20	R81	14.8	0
Alternate	2020/2/20	R82	15.1	3
Alternate	2020/2/20	R83	17.1	2
Alternate	2020/2/24	R71	28.7	3
Alternate	2020/2/24	R72	32.6	3
Alternate	2020/2/24	R74	23.4	2
Alternate	2020/2/24	R76	27.2	2
Alternate	2020/2/27	R70	34	1
Alternate	2020/2/27	R71	16.7	6
Alternate	2020/2/27	R76	16	0
Alternate	2020/2/27	R79	36.5	4
Alternate	2020/2/27	R81	18.1	1
Alternate	2020/2/27	R83	13.5	1